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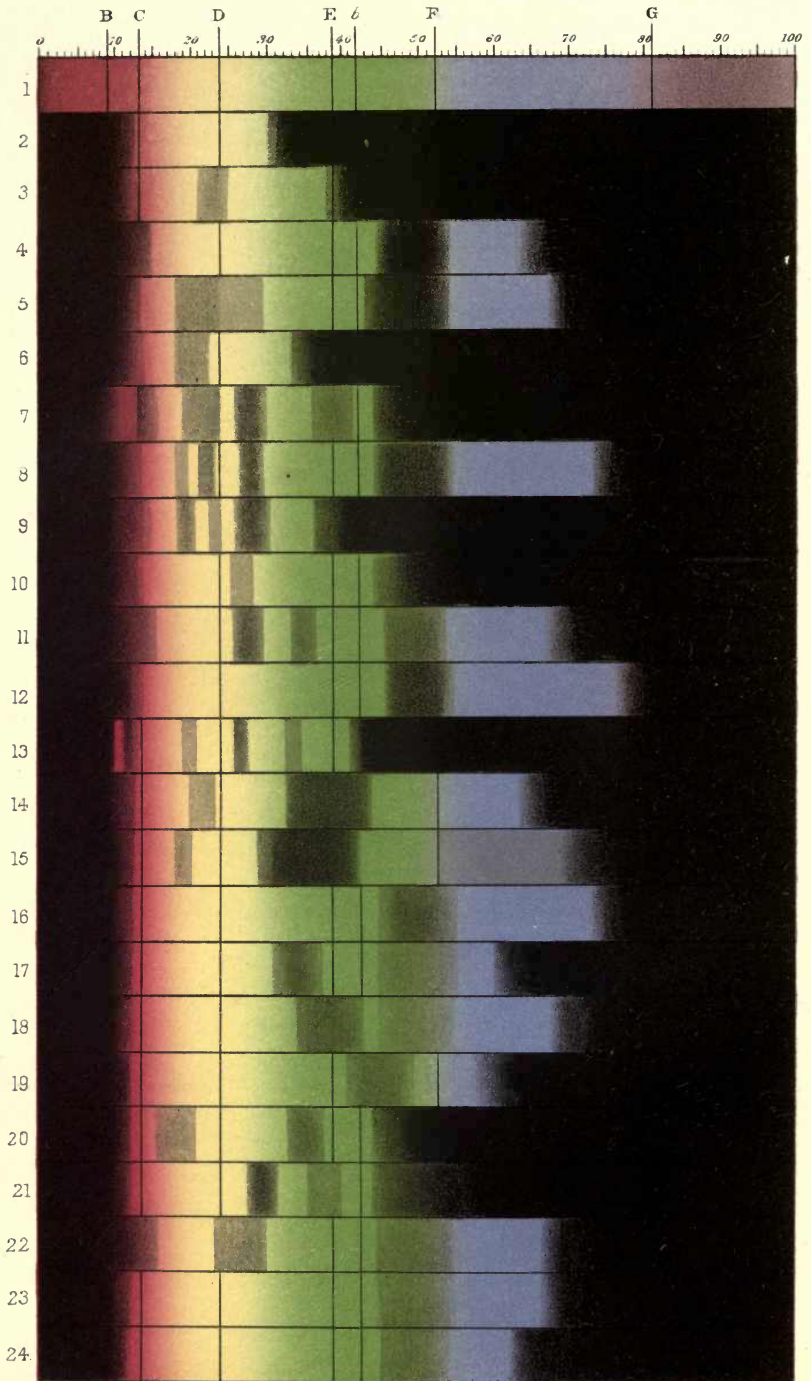
THE SPECTROSCOPE

IN

MEDICINE



CHART II.



Cha^sA. MacMunn adnat. del.

Hanhart lith.

FRONTISPIECE.

THE SPECTROSCOPE

IN

MEDICINE

BY

CHARLES A. MACMUNN, B.A., M.D. UNIV. DUB.

WITH

THREE CHROMO-LITHOGRAPHIC PLATES OF PHYSIOLOGICAL AND
PATHOLOGICAL SPECTRA AND THIRTEEN WOODCUTS



LONDON

J. & A. CHURCHILL, NEW BURLINGTON STREET

1880



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PREFACE.

THE Spectroscope seems likely to be of almost as great use in Medicine as it has already proved in terrestrial, solar, and stellar Chemistry. Hitherto the great hindrances to its use have been erroneous ideas as to the difficulties which seem to surround this method of investigation; such ideas having arisen from want of a simple treatise on the instrument and its applications to Physiology and Pathology.

In the following pages such information will be found, and the publication of this little book will, I trust, lead to a more extended use of the spectroscope, which has already done good work in Physiology and Pathology, and has corrected many erroneous ideas regarding various animal pigments.

I take this opportunity of thanking Mr Lawson Tait, Dr Saundby, and other medical friends who have kindly sent me pathological fluids for examina-

tion, also Mr Browning for his kindness in lending me the blocks of the woodcuts, Messrs Hanhart for the admirable way in which they have executed the plates, and last, but not least, Messrs J. and A. Churchill for the courteous manner in which they have met my requirements.

CHAS. A. MAC MUNN.

WOLVERHAMPTON ;

December, 1879.

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CHART I.

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2. Strong solution.
3. Weaker solution.
4. Shows the two oxyhæmoglobin bands.
5. The solution diluted sufficiently to show only one band.
6. Reduced hæmoglobin.
7. Carbonic-oxide hæmoglobin.
8. Sulphæmoglobin.
9. The same oxygenated.
10. Methæmoglobin.
11. Blood treated with nitrite of amyl and alcohol.
12. Acid hæmatin (alcoholic solution).
13. Alkaline hæmatin (alcoholic solution).
14. Blood treated with alcohol and ammonia.
15. Reduced or deoxidized hæmatin.
16. Sulphate of cruentin.
17. Five-banded alkaline cruentin.
18. Neutral cruentin in chloroform.
19. Hydrochloric product from neutral cruentin.
20. Reduced cruentin.
21. Blood treated with cyanide of potassium or with hydrocyanic acid.
22. The same deoxidized.
23. Solution of blood treated with solution of bromine.
24. The last reduced with ammonium sulphide.

All the above are mapped from the microspectroscope.

CHART II.

SPECTRUM

1. Solar spectrum with Fraunhofer's lines.
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3. Alcoholic extract of human bile.
4. Diluted solution of human bile treated with hydrochloric acid.
5. Bile treated with nitric acid, and the precipitate dissolved in boiling absolute alcohol.
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These spectra were reduced from Dr Thudichum's maps for the purpose of comparison.

SPECTRUM

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24. Ash of human body, showing bright-line spectra of the metals present.

EXPLANATION OF THE INTERPOLATION CURVE FOR THE CALCULATION OF WAVE-LENGTHS (p. 32).

By means of a curve about four times larger than that figured, the readings of any scale can easily be reduced to wave-lengths.

Along the top line is printed the scale of the instrument, and along the right hand line is the scale of wave-lengths. As many Fraunhofer's lines as possible, the wave-lengths of which are known, are measured on the scale of the spectroscope, and mapped on the paper; a curve is then drawn through these points. The method of calculating the wave-lengths of unknown lines or bands by means of this curve is fully described in the text and in Appendix I.

For the sake of clearness only a few Fraunhofer's lines are mapped in the plate, but the accuracy of the method is increased by having a great number of lines mapped, and by having the curve drawn as uniformly as possible.

Only the large squares are represented, which were square inches in the original, the smaller squares being square tenths of an inch.



THE

SPECTROSCOPE IN MEDICINE.

CHAPTER I.

THE PRISM AND ITS ACTION ON LIGHT.—CONSTRUCTION OF A
SPECTROSCOPE.—THE VARIOUS KINDS OF SPECTRA.

The Prism.—We may define a prism in Optics as a transparent body having two plane faces not parallel to one another; or we may define it for our present purpose as a wedge-shaped piece of glass; or, as a medical writer puts it, "a triangular rod of glass." Euclid defines a prism as a solid figure bounded by five surfaces, two of which are triangles, equal, similar, and parallel to each other, while the other three are rectangles. The two parallel, equal, and similar, triangles have nothing to do with the *optical* properties of the prism, so we may leave them out of the question. We are only concerned with two of the rectangular surfaces; the angle formed by the meeting of these two surfaces or faces is called the *refracting angle*, or the *angle* of the prism. If we look at a lighted candle through a glass prism, keeping the refracting edge of the prism uppermost, we cannot see

the candle until the prism has been raised to a certain height, the height depending on the angle of the prism. The prism has bent the light of the candle out of its course, in other words, in its course through the former, the light has suffered refraction or deviation; but, in addition, the candle appears surrounded with a fringe of colours, so that the prism has not only refracted the light, but also split it into its component colours. This splitting up of the light into its component colours is called dispersion.

About 200 years ago Newton made the discovery of the action of the prism upon sunlight in the following manner:—In the shutter of a darkened room he made a small, round hole; in the path of the sunlight which streamed in through this small hole, he placed a glass prism, and when the base of the prism was turned upwards, he found on a white screen placed opposite the shutter a coloured band composed of seven coloured discs, with their edges overlapping. Beginning at the lowest end of the coloured band was a red disc, then orange, yellow, green, blue, indigo, and violet discs. In this way, and by the recomposition of the spectrum, Newton discovered that white light is split up by the prism into its component colours; he also found that these different colours were not all bent equally by the prism; so that “the light of the sun consists of rays of different refrangibility.” Red light is not so far deviated from its direction as the blue or violet. This coloured band on the screen was the spectrum of sunlight, but in consequence of the overlapping of each other by the coloured discs it was not

a pure spectrum, the colours were mixed at the edges of the discs. In 1802, Dr Wollaston discovered that if a slit were made in the shutter instead of a round hole, the spectrum of sunlight, instead of being composed of a number of coloured discs, was now a band of pure colours, each colour being free from admixture with the one next to it. Moreover, he found that this coloured band was not continuous, as Newton described it, but interrupted here and there by *fine black lines*. In 1814, Fraunhofer,* a German optician, discovered these lines independently, and mapped 576 of them, calling the most prominent A, B, C, D, E, F, G, H, which lines he used as marks for comparison. He also found that the distances of these lines from each other may vary according to the nature of the substance composing the prism; thus their relative distances are not the same in prisms of flint-glass, crown-glass, and bisulphide of carbon, but they always occupy the same position relatively to the colours of the spectrum. Kirchhoff and Ångström have now mapped more than 2000 Fraunhofer lines.

In 1830, Simms, an optician, made an improvement in the construction of the spectroscope by placing a lens in front of the prism, so arranged that the slit was in the focus of the lens. This lens turns the light after it has passed through the slit into a cylindrical beam before it enters the prism. Another lens was also introduced by him which receives the circular beam emerging from the prism and compels it to throw an image of the slit, which may be magnified at

* Born in 1787 at Straubing, a small town in Bavaria.

pleasure for each ray. The lens between the prism and the slit is called the *collimating* lens. Thus the following are the essential parts of a chemical spectro-scope :

1st. A slit, the edges of which are two knife-edges of steel or other metal very truly ground and exactly parallel to each other, and in a direction parallel to the refracting edge of the prism, to admit a pencil of rays.

2nd. A collimating lens; a convex lens with the slit at its principal focus, which renders the rays parallel before entering the prism. The tube in which this lens is placed is equal in length to the focal length of the lens, and is called the collimator.

3rd. A prism of dense glass, in which the parallel rays are refracted and dispersed. It must be placed so that all the rays are refracted through it with approximately minimum deviation.

4th. An observing telescope, constructed like an astronomical refractor of small size, and placed so that the rays shall traverse it after emerging from the prism. It must be focussed as if for viewing a distant object, because rays of a given refrangibility from a given point of the slit are parallel before entering it (Everett). Such are the essentials of a one-prism chemical spectro-scope. In a direct-vision instrument such as the Sorby-Browning microspectroscope, the construction is somewhat different, which will be explained when we come to describe this instrument.

When various sources of light are examined with a combination such as has been described, we find that spectra can be classed under four heads.

1st. *Continuous spectra*, yielded by all white-hot fluid and solid bodies with a few exceptions, a coloured band passing from red through orange, yellow, green, blue, indigo,* and violet. This class of spectra is given when we illuminate the slit of the spectroscope with gaslight, candlelight, lamplight, magnesium light, limelight, and the electric light, &c.; white-hot platinum, and iron in a state of fusion, also give continuous spectra.

2nd. *Spectra which present bright lines and striæ on a dark back-ground*.—This class is yielded by glowing vapours and gases, and each chemical element has its own characteristic spectrum. In discussing the application of bright-line spectra to medicine they will be considered more in detail.

3rd. *Absorption spectra*.—When certain coloured vapours, such as those of nitrous acid and of iodine, are placed between the source of light and the slit of the spectroscope, a number of dark lines appear at right angles to the length of the spectrum; the former gives, according to Brewster, 2000 lines, sparingly present in the red part of the spectrum, but more closely arranged toward the violet end. On the other hand the violet-coloured vapour of iodine gives a number of dark lines in the orange, yellow, and green, while the violet part of the spectrum is free from them.

According to Wüllner this spectrum is the exact converse of that got by examining glowing iodine vapour, as the latter, which is obtained by examining the reddish-yellow light of a hydrogen flame, saturated with iodine vapour, is characterised by the presence of bright lines at

those points where the absorption spectrum appears dark. The vapour of nitrous acid stops certain kinds of rays from the light-source traversing it, especially the violet ones, and the iodine vapour, being almost opaque for yellow and green rays, stops most of these rays. Consequently the continuous spectrum which would have been got from the source of light, if it were allowed to fall on the slit without having to traverse either of these vapours, is interrupted by black lines, spaces of darkness, caused by the stoppage of the rays, corresponding to the dark spaces, by the vapours of nitrous acid or of iodine.

The colours of solids and fluids result from their being capable of absorbing certain rays of white light, and of reflecting others; an object is red because it absorbs all rays except the red, which it reflects, and so it appears to be of a red colour. A white object reflects all the rays of white light and so appears to have no colour; while an object is black when it absorbs most or all of the light falling on it. Again, the different colours of *transparent* solids and fluids result from their capability of absorbing certain rays. A solution of permanganate of potassium transmits the red and blue-violet parts of the spectrum unaltered, while in the yellow and green are black bands, the colour of the fluid itself is reddish-violet, and it transmits the red and violet parts of the spectrum. Again, chlorophyll or the colouring matter of leaves gives, when in alkaline solution, a well-marked absorption-spectrum, in the middle of the red a black band between B and C, three feeble absorption striæ in orange-yellow

and green, while the violet and indigo colours are entirely shaded. Sometimes absorption bands appear faint and hazy, at other times sharp and clearly defined. The method of studying absorption spectra will be referred to further on.

4th. The next class* of spectra is that which we get when we examine sunlight with the spectroscope. This spectrum is composed of the red, orange, yellow, green, blue, indigo (or more properly ultramarine), and violet colours, forming the band which Wollaston got by transmitting the ray of sunlight through the slit and the prism, as before described, so far resembling the continuous spectra of white-hot solids and fluids; but, in addition, these colours are here and there interrupted by the presence of black lines, some of them of exceeding fineness, all these lines being placed at right angles to the length of the spectrum. Some of these lines (which were described by Fraunhofer, as mentioned before, and therefore called Fraunhofer lines) are better marked than the rest, and as they always occupy the same position relatively to the colours of the spectrum, they are taken as marks, to which the position of absorption bands is referred. The first Fraunhofer line A, is in the red, so also are *a*, B, C; D is in the orange-yellow, E and *b* in the green, F between blue-green and blue, G in the violet-blue, and H in the violet.

Fraunhofer discovered that the bright yellow sodium line occupies the same position in the spectrum as Fraunhofer's line D, and the same experiment is easily

* The spectrum of starlight belongs to the same class.

performed by any one who possesses a spectroscope, for by directing sunlight on the slit of the spectroscope, the Fraunhofer lines appear, and then causing the light from a spirit lamp or Bunsen burner, which has in its flame a salt of sodium, to fall upon the right-angled prism covering half of the slit, it will be at once evident that the D line of the solar spectrum is coincident with the bright yellow line of sodium; and by a method which, in principle, is the same as this, it has been shown that most of the lines in the solar spectrum occupy the same position in the spectrum as the bright lines of various chemical elements; in this way the foundation of solar and stellar chemistry has been laid. But if the Fraunhofer lines are the lines of the incandescent vapours of elements burning in the sun, why are they black? Why is the D line not yellow? In 1859 Kirchhoff discovered that vapours in a comparatively cool state had the power of absorbing the light emitted by the same vapours in an incandescent state; or, putting the law in more accurate language, *every gas and every vapour absorbs exactly those kinds of rays which it emits when in the glowing condition, whilst it permits all other kinds of rays to traverse it with undiminished intensity.* Two experiments prove this law. (1) If the light from the limelight be allowed to fall on the slit of a spectroscope, and then a flame coloured by a salt of sodium be interposed between the light and the slit, a black line appears in the position of the sodium line; or, (2) If* a piece of sodium be burned in an iron spoon, and it is then ignited, at first we get the yellow

* Suffolk.

line only, but as the sodium gets hotter and begins to glow we get a continuous spectrum. Afterwards the vapour surrounding the burning sodium absorbs the yellow line and a black one appears instead.* Although the causation of these Fraunhofer lines is explained in a great number of books on Physics, &c., yet I have thought it right to give a brief epitome of it, as a great deal of confusion exists in people's minds with regard to this most important subject. The Fraunhofer lines are of the greatest importance in the study of absorption bands, because the principal ones, as mentioned above, are used as marks to indicate certain parts of the spectrum, with the position of which the position of absorption bands is compared (and from which measurements can be taken), so that we may be able to state that a certain absorption band coincides with C or D, or other Fraunhofer line.

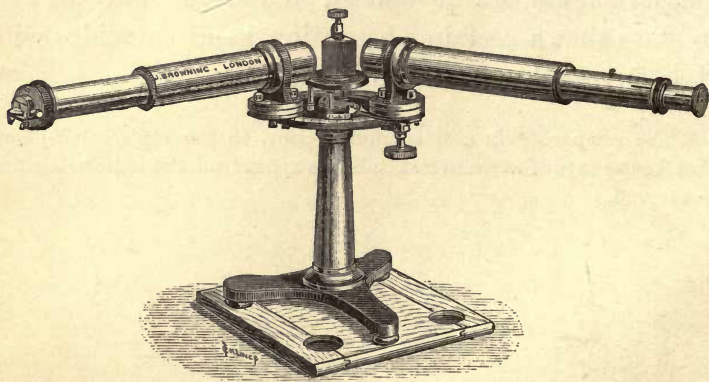
* The *comparatively cool* sodium vapour, in the sun's atmosphere, absorbs the rays of *incandescent* sodium vapour from the sodium burning in the sun.

CHAPTER II.

INSTRUMENTS, AND METHODS OF USING THEM, ETC.

Chemical Spectroscopes.—The one-prism chemical spectroscope is shown in fig. 1, and is seen to consist of *a slit* carried in the left-hand tube, which tube is called the *collimator*, at the other extremity of which is the *collimating lens*, of a

FIG. 1.

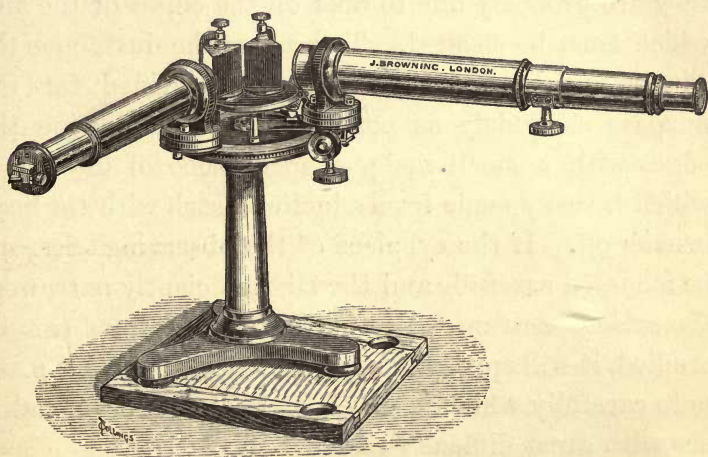


prism, and an *observing telescope*; the small wheel represented beneath the right-hand tube is for clamping it in any required position. The right-hand tube has attached to it an arm carrying a vernier, which moves on a graduated arc of a circle divided into degrees and minutes; the use of this will be referred

to further on. The slit is furnished with a right-angled reflecting prism, by means of which two spectra can be seen in the field of view at the same time. This instrument can be used for taking the refractive and dispersive powers of solids and liquids if necessary.

Fig. 2 represents a chemical spectroscope of two prisms, which is preferred for the study of bright-line spectra, as it possesses more dispersive power than a one-prism spectroscope, but for medical purposes the

FIG. 2.



one-prism spectroscope is to be preferred, as it is amply sufficient for the study of the few bright-line spectra which have to be studied; and moreover one prism is better than two—on account of its small dispersion—in the study of absorption bands.

How to observe spectra with the one-prism spectro-scope.—Having screwed the tube carrying the slit into the ring fixed on the divided circle and the

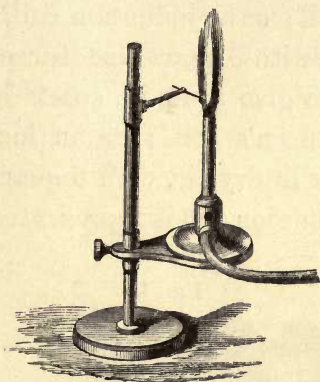
observing telescope into the ring provided with the movable index, remove the cap which covers the slit, direct the slit to the window, and turn the telescope with the index round the graduated arc until a spectrum appears. Cover the prism and those parts of the tube next it with a piece of black velvet to exclude surplus light, taking care that the velvet does not interfere with the progress of the light through the various parts of the spectroscope. If now lines parallel to the *length* of the spectrum are apparent they are probably due to dust on the edges of the slit, which must be cleaned. To remove the dust, open the slit by means of the small screw provided for the purpose as widely as possible, and gently wipe the edges with a small wedge-shaped piece of dry wood, which is easily made from a lucifer match with the head broken off. If the eyepiece of the observing telescope be focussed carefully and the slit sufficiently narrowed, the solar spectrum with the Fraunhofer lines can be studied, it will appear as represented in Chart I, Sp. 1; note carefully which is B, C, D, E, *b*, F, G. A and *a* are with great difficulty seen, so also is H.

Now illuminate the slit with the light from an ordinary gas-burner, turning the *edge* of the flame to the slit; note there are no longer Fraunhofer lines, nothing but the continuous spectrum of gaslight.

To observe a bright-line spectrum.—Take a Bunsen burner mounted on a suitable stand, such as that represented in fig. 3, turn on the light-flame by stopping up the holes at the bottom of the tube, in the burner shown here there is a ring at the bottom provided with four

apertures ; by turning the tube round these are closed, and we get a visible flame ; now bring this within a few inches of the slit, observe the continuous spectrum. Now turn the tube so that the holes at the bottom are open. Take a piece of platinum wire, about the thick-

FIG. 3.

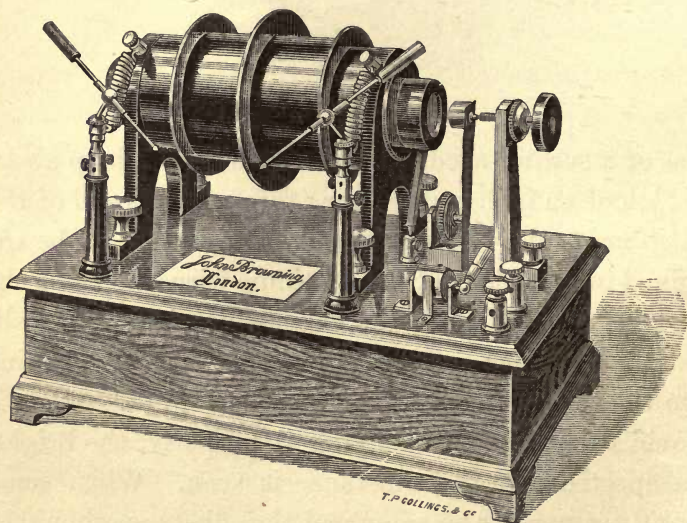


ness of a sewing-needle, bend the end into a loop about the $\frac{1}{8}$ th of an inch in diameter ; fuse a small bead of the salt (one of the alkalies or alkaline earths) to be examined into the loop of the platinum wire, and bring the bead into that edge of the flame which is next the slit and a little below the level of the latter. On looking into the telescope tube, if it has not shifted, which is provided against by clamping it properly, the bright-line spectrum of the substance is seen. When small quantities have to be examined the substance should be dissolved, and a drop of the solution, instead of a solid bead, be used on the platinum wire. The *chlorides* of the alkaline earths, and *carbonate* of sodium and *ferro-*

cyanide of potassium are the best salts for examining the bright-line spectra of these metals.

To observe the bright-line spectra of the Heavy Metals.—To study the bright-line spectra of the heavy metals, since they cannot be volatilised at the temperature of the Bunsen flame, we require: “either an induction coil, to give a $2\frac{1}{2}$ in. spark in dry air, with 1 quart size Bunsen’s cell; or an induction coil, to give a $3\frac{1}{2}$ in. spark in dry air, with 3 quart size Bunsen’s cells; or an induction coil, to give a $4\frac{1}{2}$ in. spark in dry air, with 5 quart size Bunsen’s cells; or an induction coil, to give a 6 in spark in dry air, with 6 quart size Bunsen’s cells.” The induction coil is represented in fig. 4; this

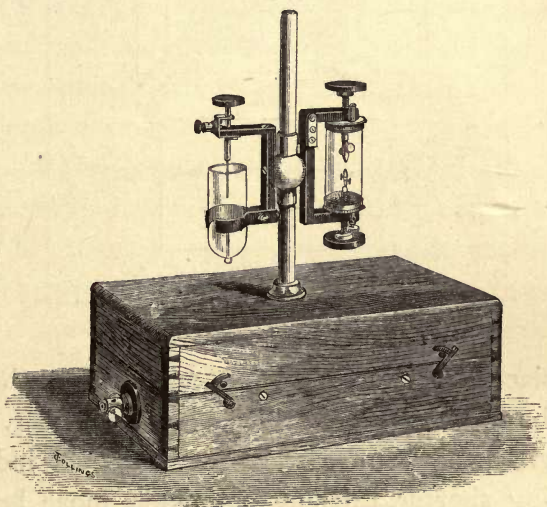
FIG. 4.



coil will also be required for observing the bright-line spectra of gases enclosed in induction tubes. “When

the trouble of charging Bunsen's cells is objected to, or it is desirable to avoid the nitrous fumes they give off, bichromate batteries may be employed. These batteries are very cleanly, but not nearly so powerful as the Groves' or Bunsen's batteries, so that the coils will not work with their full power when they are used. A bichromate battery can be arranged so that, by using a winch, the elements may be removed from the exciting solution at pleasure. These batteries may be used many times without re-charging." Instead of using a Leyden jar to increase the temperature of the spark, a spark condenser, such as that shown in fig. 5,

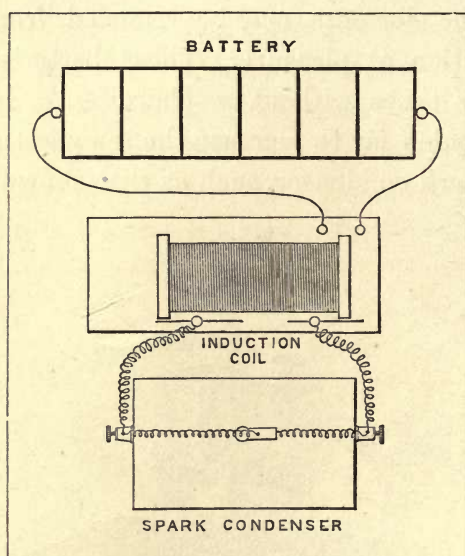
FIG. 5.



may be used. Fig. 6 shows how it is used; the method of using it is thus described by Mr Browning:—"Connect the wires from the battery with the two clamp screws at one end of the induction coil, then

carry a fine wire from each of the terminals of the coil (the points from which the sparks are given), one to each clamp screw at the opposite ends of the spark condenser. If the commutator of the coil be now turned on the spark will pass between any pieces of

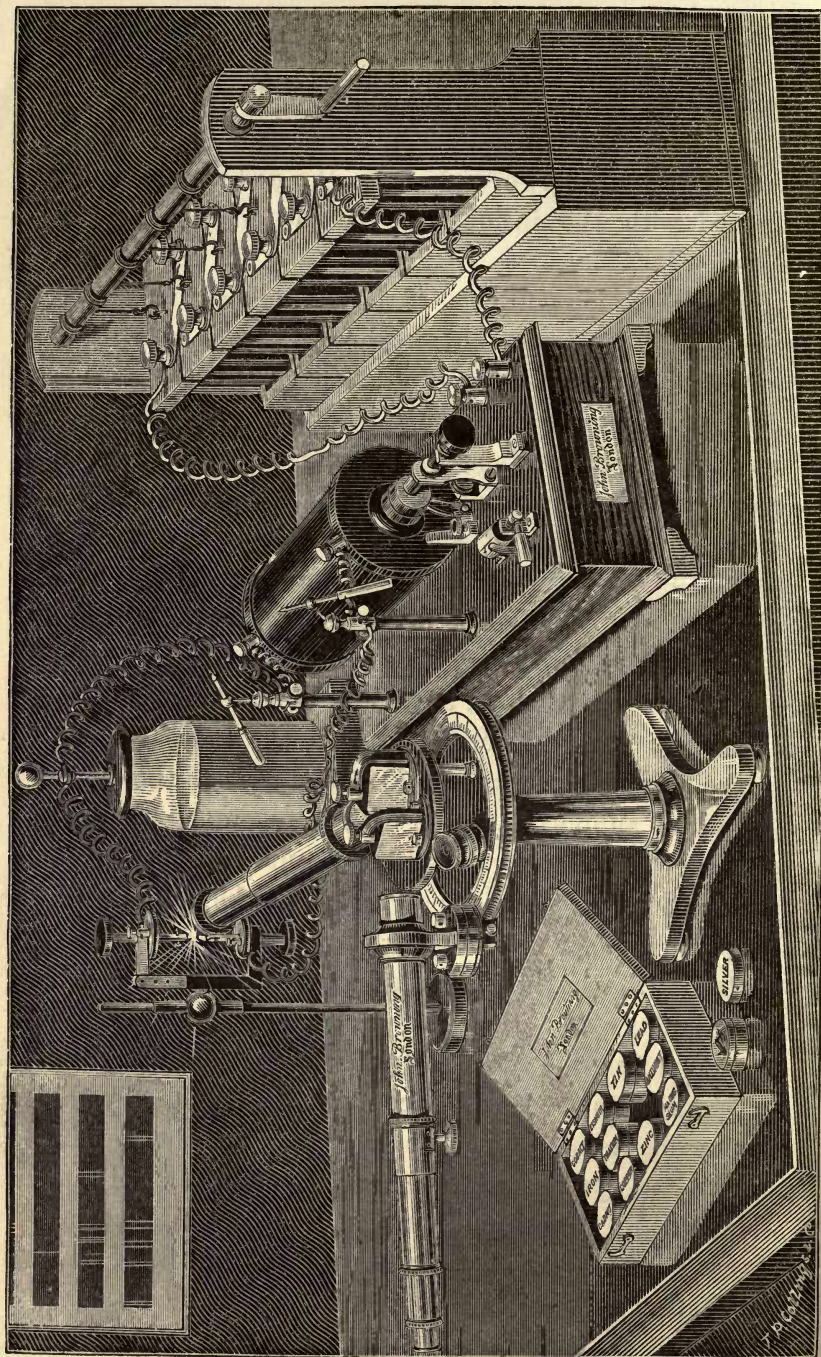
FIG. 6.



metal placed in the two pairs of tweezers on the insulated ebonite support of the spark condenser. This spark will be very different from that of the induction coil, being shorter, thicker, and much more brilliant. A spark given through the condenser from $\frac{1}{10}$ th to $\frac{1}{8}$ th of an inch long is best adapted to give the spectra of the metals in the spectroscope." The diagram, fig. 6, will perhaps show more clearly than any description how the connections between the battery, spark con-

denser, and coil are to be arranged. "When a Leyden jar is used the connections are arranged in a similar manner, but the two wires from the coil must be connected with the inside and outside coating of the Leyden jar."

The induction coil used in obtaining the bright-line spectra of the heavy metals should give a spark two inches long in dry air, and unless a spark condenser is used a Leyden jar must be introduced into the circuit for the purpose of increasing the temperature of the spark. "Two small pieces of the metal of which the spectrum is required should be placed in forceps attached to the terminals of the induction coil. The pieces of metal should be brought within one eighth of an inch of each other. The spark should pass in a vertical line parallel to, and in front of, the slit. The Leyden jar must be connected with the induction coil in the following manner:—Attach a wire to the metal rod which supports one pair of forceps on the terminal of the coil, and carry this to the outside covering of the Leyden jar. A second wire should be attached in a similar manner to the other pair of forceps, and connected with the inside covering of the Leyden jar. This suffices to bring the jar into circuit." The accompanying fig. 7 from Mr Browning's book, 'How to Work with the Spectroscope,' will show clearly the arrangement of apparatus required to obtain the bright-line spectra of the heavy metals, and I am indebted to the same source for the directions given above. In the chapter which treats of the application of bright-line spectra to medicine, the



method which Dr Thudichum employed in obtaining the bright-line spectra of morbid gases will be referred to.

The method of getting the absorption spectrum of a fluid.—Illuminate the slit with the edge of the gas-flame, or, if this cannot be obtained, with the light of a paraffin lamp, then place in a suitable holder (mine was made from a retort stand and a test-tube holder) a test tube containing the solution to be examined between the slit and the light, and observe the spectrum; examine the solution in test tubes of different diameters until the best effect is obtained. If the solution be too dilute to give an absorption-spectrum examine it in a deeper layer, or, if possible, use a stronger solution.

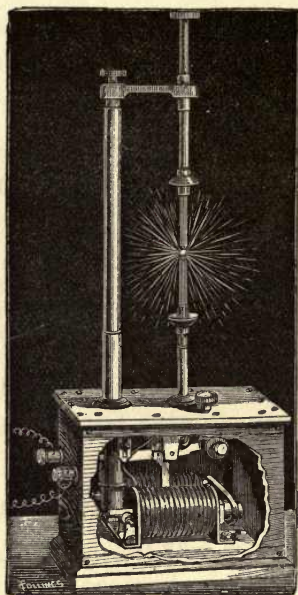
In examining some solutions the electric lamp is required. Fig. 8 represents a convenient lamp; twenty quart cells are used with it.

How to map spectra observed with the one-prism chemical spectroscope.—(1) *By means of the nonius and arc.*—Most one-prism spectroscopes are now provided with a vernier (*vide antea*, fig. 1) and graduated arc. Suppose we want to make a map of the Fraunhofer lines. In the eye-piece of the observing telescope are two cross wires; turn the eye-piece until the cross is in this direction, X, and make the point of *intersection* of the wires correspond (by moving the telescope along the graduated circle) to the first visible Fraunhofer line, note the reading, then move the telescope until the intersection of the X is over another line, and take the reading as before, and so on for the other lines. From



these several readings, by means of a scale of equal parts, a map of the solar spectrum is easily constructed. Bright-line and absorption spectra are mapped in the same manner. The advantage of this method is, that

FIG. 8.



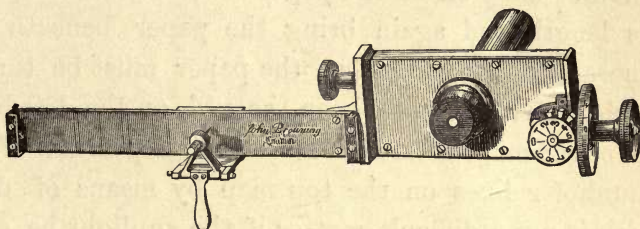
the readings are given in angular measurements, but its disadvantage is the time spent in taking the measurements.

(2) *By means of the photographed millimètre scale.*—Some one-prism spectroscopes are provided with a third tube carrying a photographed scale on glass; the image of which, by means of a lens, is thrown on to one of the surfaces of the prism, by which it is reflected into the observing telescope, and is seen to divide the

spectrum into a certain number of parts. The illumination of this scale necessitates the use of a second light, but it has the great advantage of enabling the readings to be taken in a very short space of time.

(3) *By means of spectrographs.*—To those who can afford it, Colonel Campbell's spectrograph is a valuable addition to the spectroscope; it is represented in fig. 9.

FIG. 9.

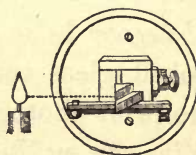


To use it, a strip of smoked glass is placed in the frame attached to the side of the micrometer; the tube of the micrometer is then placed in the eye-draw of the telescope of a spectroscope; the cross wires of the micrometer are now brought on to one of the lines in the spectrum by turning the micrometer screw. A line is drawn on the smoked glass with the small ruling machine on the left hand in the figure. This operation is repeated for as many lines as it is thought necessary to map, *the screw being always turned in one direction*. The smoked glass can be varnished and used as a negative to print photographs from.

(4) *By means of the camera lucida.*—The simplest and the readiest method of mapping bands is, in my opinion, that which I described for the first time in the 'Dublin Journal of Medical Science,' 1877, viz. by means of the camera lucida. A camera-lucida prism is

made to fit the eye-piece of the observing telescope. To map the Fraunhofer lines by this method proceed as follows:—Having illuminated the slit with sunlight, and focussed the observing telescope until the lines appear distinctly, slip the camera lucida over the eye-piece and with a pencil mark the length of the spectrum on a sheet of white paper placed on the table beneath the camera. Rule off on the paper several blank maps of this length and again bring the paper beneath the camera, remembering that the paper must be turned upside down so as to keep the red on the left-hand side of the maps afterwards; mark the position of the Fraunhofer lines on the top map by means of dots, which is not a difficult matter if the sunlight be sufficiently strong; and draw lines through the dots. We have now a solar map from which we can construct any number afterwards. To map an absorption spectrum:—Having placed the test-tube containing the solution before the slit and illuminated it with a strong enough light, turn the right-angled reflecting prism so that it shall cover half the slit (fig. 10); place a Bunsen

FIG. 10.



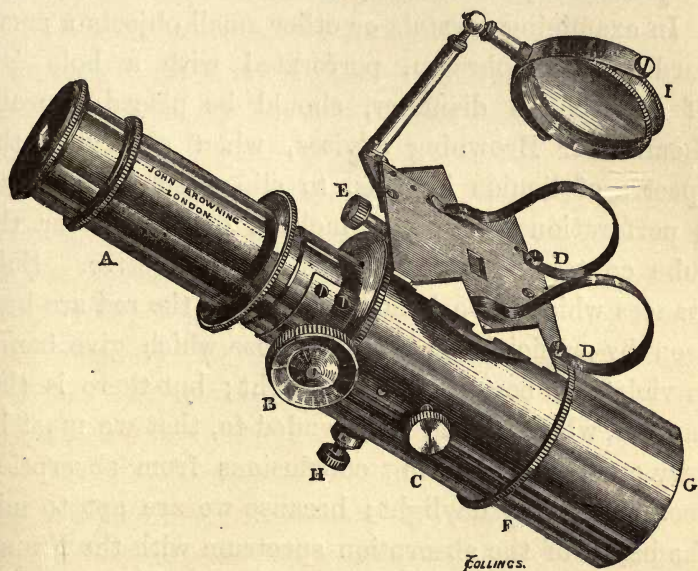
burner with a salt of sodium introduced into its flame, so that its light may fall on the reflecting prism. On looking into the spectroscope two spectra are visible, one the absorption spectrum, the other the bright

yellow line of sodium ; now bring the blank solar map (turned upside down or in the position in which it was, when the Fraunhofer lines were originally mapped on it) beneath the camera, and move it from side to side *until the yellow sodium line covers exactly Fraunhofer's line D*, then with a pencil mark the position of the absorption bands on the map, and also the extent of the shading on the red and violet side. The map can be afterwards completed with Indian ink ; it can be constructed in a much shorter time than I have taken to describe it, and must of necessity be accurate, since we always have a fixed point to start from—the sodium line. If it is thought desirable a scale can be attached to this map by laying along it a millimètre scale and marking the divisions along the top line of the map ; such a scale is useful for comparison, and also for enabling the position of the bands and their breadth to be expressed in wave-lengths ; in order that they may be so expressed we must remember to have the same number always opposite the D line.

The Microspectroscope.—In the microspectroscope we have a slit and a compound prism, but no observing telescope, the spectrum being viewed directly by the eye. The Sorby-Browning is the best microspectroscope I know, and is a very complete and beautiful little instrument. Its internal construction is as follows : The prisms it contains are those known as “*direct vision*,” they consist of two prisms of flint glass between three of crown glass, united together by means of balsam (fig. 11, p. 24). By this arrangement deviation is eliminated, while sufficient dispersion is retained. The

spectra together; the light is reflected into the square hole in the side stage by means of the mirror (I, fig. 12); the size of the aperture in the side stage is regulated by the screw (E). F is placed below the field lens of the

FIG. 12.



eye-piece. G is a tube which fits into the tube of a microscope instead of the eye-piece of the latter.

To examine an object with the microspectroscope place it on the stage and focus it with the ordinary eye-piece, then throw it slightly out of focus and substitute the microspectroscope for the eye-piece. Opaque objects can also be examined with the microspectroscope if they are illuminated by means of the bull's-eye condenser, Lieberkühn, side reflector, &c. Mr Sorby uses a binocular microscope, as the left-hand

tube can be used as a finder; for my part I prefer a small monocular microscope, and by practice having learned the focal distances of the different lenses from the surfaces of the various fluids and solids, I never find any difficulty in getting a spectrum. In examining very small objects a high-power objective is required.

In examining crystals or other small objects a small cardboard diaphragm, perforated with a hole $\frac{1}{8}$ th of an inch in diameter, should be placed beneath them. Mr Browning advises, when observing the spectra of liquids in cells, to slip a small cap with a perforation $\frac{1}{16}$ th of an inch in diameter over the tube containing the $1\frac{1}{2}$ - or 2-inch objective. Substances which give bands or lines in the red are best seen by artificial light, while those which give bands in violet are best seen by daylight; but there is this caution, which should be attended to, that we must be very careful in drawing conclusions from absorption spectra seen by daylight; because we are apt to mix the bands of the absorption spectrum with the Fraunhofer lines; thus, if the edge of a band happens to coincide with a Fraunhofer line we are apt to imagine that the band is better defined, more abruptly shaded on one side than it really is.

Cells and tubes for the microspectroscope.—For a long time I was grievously disappointed, in working with the microspectroscope, to find that my results were vitiated by the mixture of the contents of the glass cells (invented by Mr Sorby) with the cement connecting the cells to the glass plates on which they are placed; moreover, the cells themselves were continually

coming off when solvents such as alcohol, ether, and chloroform, which are being continually used in physiological work, were put in them. I therefore invented cells for myself, and after an extended trial I can fully recommend them. They are merely flat-bottomed tubes of different diameters fitted into thin slabs of wood. Some are made from thin glass tubing carefully sealed at one extremity; the latter answer quite as well as Mr Sorby's barometer-tube cells. If necessary they can be made from barometer tubing, and their bottoms can be ground flat and polished. Even small short test tubes can also be used in this manner. Besides the fact that solvents cannot affect these tubes, they have also the advantage of being easily cleaned, and the wood in which they are set stops all extraneous light, so that we only get those rays which come through the bottom of the tube or cell. For *comparison tubes* to be used with the side stage the little flat tubes made for bouquet-holders are very convenient, and being of two different diameters they allow two depths of fluid to be examined.

For examining such fluids as bile I find glass slips with excavated cells, such as are used for mounting seeds, &c., very useful, as the shape of the excavated cell allows different strata of material to be examined.

How to map spectra from the Sorby-Browning microspectroscope.—(1) *By means of bright-point micrometer.*—If the reader will turn back to fig. 11, which represents the internal construction of the microspectroscope, the arrangement for throwing a bright

point on to the surface of the upper prism will be understood. A A is a small tube attached to the side of the larger one. At the outer part of this tube is a blackened glass plate, with a fine clear white pointer in the centre. c is a lens, which can be focussed by the studs (B B), and which produces an image of the bright point in the field of view by reflection from the surface of the prism next to the observer's eye. The micrometer screw (M) causes the slide which holds the glass plate to travel in grooves, and so the fine pointer is made to cross the whole length of the spectrum. To map the Fraunhofer lines the bright point is made to coincide with one of them, and the reading taken on the graduated wheel; the distance between this line and the next is also measured by counting the number of divisions passed through in turning the wheel, and so on; the lines are then mapped on paper by means of a scale of equal parts. This map is kept for reference. An absorption spectrum is mapped by measuring the distance of its bands from certain fixed points, such as the sodium line, &c. I must confess that this bright-point micrometer is not at all a pleasant method of mapping spectra, as it monopolises a great deal of time, and, if great care is not taken, it is apt to lead to error.

(2) *Mapping by means of photographed scale.*—Mr Browning has fixed to the side of the prism tube of my microspectroscope a tube in the position of A A, fig. 11, which contains a photographed scale, illuminated by a small mirror, and which is capable of being focussed by a small lens in the position of c, so that,

on looking into the instrument, one can see the spectrum beautifully and accurately divided into 100 equal parts, since the image of the scale is reflected to the eye from the surface of the uppermost prism. By means of this scale readings can be made at once, the only precaution necessary is to see that D, or the sodium line if D cannot be got, always stands at the same number on the scale. To map absorption spectra measured on this scale is exceedingly simple, all we have to do is to lay down a line as many millimètres long as there are divisions in the scale, and mark the position of the bands on this line. Of course, as in the case of the bright-point micrometer, a map of the solar spectrum has to be first made. Another great advantage of this scale, besides the rapidity with which measurements can be made with it, is this, that it enables us to record certain data from which maps can at any future time be constructed. Thus we can record a spectrum somewhat in this manner:—

Extent	10 to 70	
First band . . .	15 to 18	. . . (a)
Second band . .	25 to 30	. . . (β)

Any one who has worked with the spectroscope can appreciate the convenience of this method.*

The irrationality of dispersion and the necessity of reducing the readings of a spectroscope to wavelengths.—Besides the *refraction* spectrum, or that obtained by refraction through a prism, there is a *diffraction* spectrum. If a piece of glass is ruled with

* There are various other methods of measuring, which will be found described in the various papers mentioned in Appendix II.

parallel equidistant scratches by means of a dividing engine and diamond-point at the rate of some hundreds or thousands to the inch, we find, on looking through it at a slit or other bright line (the glass being held so that the scratches are parallel to the slit), that a number of spectra are presented to view, ranged at nearly equal distances at both sides of the slit, and the spectra, if the experiment is properly made, will show Fraunhofer's lines. The spectra may be used with a telescope focussed on the plane of the slit. This piece of glass is called a grating. "A grating for diffraction experiments consists essentially of a number of parallel strips, alternately transparent and opaque." Such spectra are of use in furnishing—(1) A uniform standard of reference in the comparison of spectra. (2) The most accurate method of determining the wave-lengths of the different elementary rays of light (Deschanel).

Ångström calculated by means of a diffraction spectrum the wave-lengths for several hundred of the dark lines of the solar spectrum; he used gratings about $\frac{3}{4}$ of an inch square, some of which had 4500 lines ruled to the inch.

The diffraction spectrum, from the ease with which wave-lengths can be calculated from it by means of a simple formula which will be found in Deschanel's 'Natural Philosophy,' pp. 1027-8, is always used for this purpose, and the wave-lengths thus calculated are constant numbers.

In refraction spectroscopes the relative distances between the Fraunhofer lines are found to vary accord-

ing to the material composing the prism (irrationality of dispersion); thus the following table gives the approximate distances between B, D, E, F, G in different prismatic spectra, and in the standard diffraction spectrum, the distance from B to G being in each case assumed equal to 1000 :—

	Flint glass, angle = 60°.	Bisulphide of car- bon, angle = 60°.	Diffraction or dif- ference of wave length.	Difference of wave frequency.
B to D.....	220	194	381	278
D to E.....	214	206	243	232
E to F.....	192	190	160	184
F to G.....	374	410	216	306

Thus it will be seen that the relative distances between the Fraunhofer lines are dependent on the nature of the material composing the prism, *so that no two arbitrary scales give the same reading*, therefore all readings should be reduced to wave-lengths, which is not by any means as difficult a matter as it is supposed to be.

Ångström has calculated the wave-lengths of light in tenth-mètres, *i.e.* in terms of a unit of which 10^{10} make a mètre, hence the name; and he has arrived at the following numbers for the wave-lengths corresponding to the principal Fraunhofer lines :

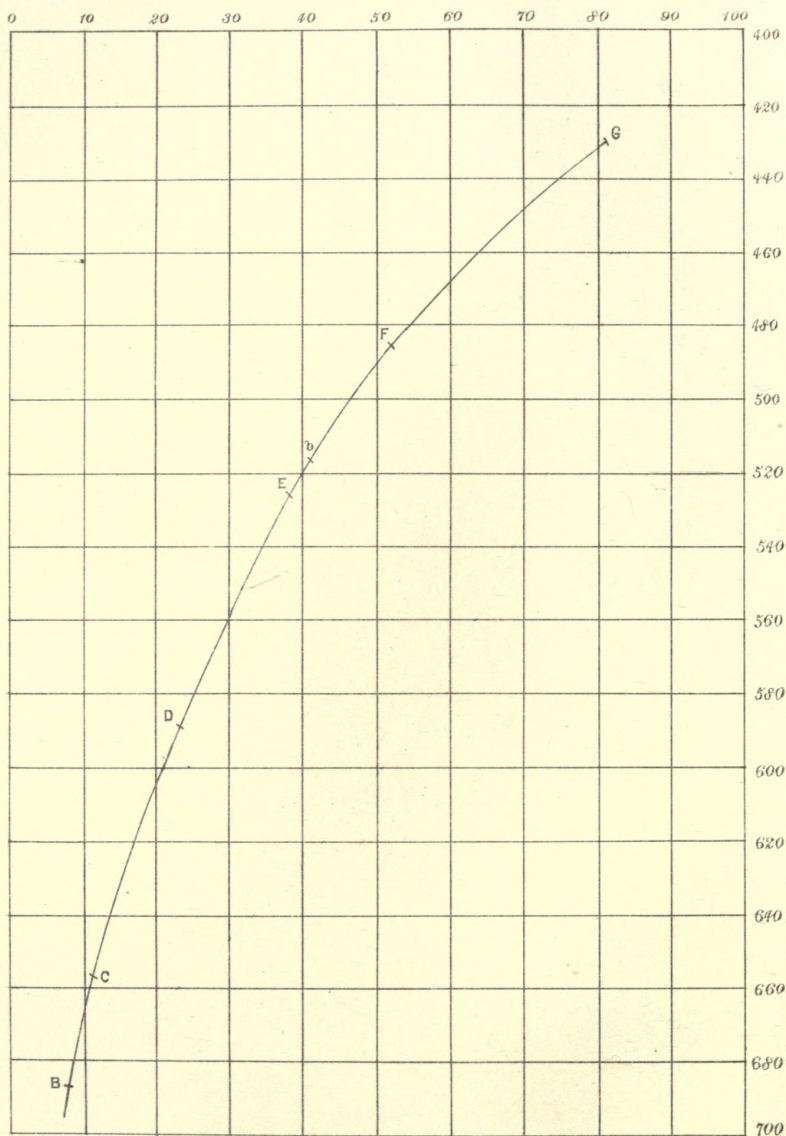
A	.	.	.	7604·00
B	.	.	.	6867·00
C	.	.	.	6562·01
D	.	.	.	5892·12
E	.	.	.	5269·13

F	.	.	.	4860·72
G	.	.	.	4307·25
H ₁	.	.	.	3968·01
H ₂	.	.	.	3933·00
Or in millionths :				
A	.	.	.	760·4
B	.	.	.	686·7
C	.	.	.	656·2
D	.	.	.	589·2
E	.	.	.	526·9
F	.	.	.	486·0
G	.	.	.	430·7
H ₁	.	.	.	396·8
H ₂	.	.	.	393·3

For calculating absorption bands in wave-lengths* the decimal may be neglected. Having now got the wave-lengths of the principal Fraunhofer lines, the wave-length of any line, or the wave-lengths corresponding to the edges or to the centre of an absorption band, can be easily calculated.

The easiest method, and the one which I shall adopt here, is that of graphical interpolation. A piece of paper ruled into square inches and tenths has a scale of wave-lengths ruled off along one edge, and the edge at right angles to this has a scale corresponding to the scale of the instrument marked on it. The value of the Fraunhofer lines on the scale of the spectroscope is observed, and by a reference to the above numbers, their value in wave-lengths, they are then marked

* The wave-length corresponding to *a* is 718·5, and to *b* 517·2.



INTERPOLATION CURVE,
FOR THE CALCULATION OF WAVE-LENGTHS.

in their proper position on the scale with ++. A curve is then drawn through these marks *as uniformly as possible*. When a band or bright line has to be mapped all that is necessary is to take its reading on the scale, then, knowing between what lines it is placed, we find its position on the curve, opposite which its wave-length is printed on the right hand edge. The accompanying diagram is a reduction from one four or five times its size, and will clearly explain the method, for which I am indebted to Watts's 'Index of Spectra.' Paper suitable for calculating wave-lengths in this way, ruled in square inches and tenths, is sold by Letts and Co. The accuracy of the method depends upon the number of Fraunhofer lines marked down, the uniformity of the curve, and upon the size of the scale through which the curve is drawn.*

* Mr. Sorby thinks that it is only necessary to express wave-lengths in millionths of a millimètre (see the second column of figures) in the case of absorption bands, and he gives the wave-length corresponding only to the centre of a band. If the wave-length of the centre of a band is only required we must take the reading of its centre *only* on the scale of the spectroscope.

CHAPTER III.

THE APPLICATION OF THE STUDY OF BRIGHT-LINE SPECTRA
TO MEDICINE.

METALS may be present in the body either as normal ingredients, or they may have been introduced into it.* Those metals which are normal ingredients are potassium, sodium, calcium, magnesium, copper, and iron. Sometimes, though their presence is not essential, lithium, rubidium, caesium, and manganese, are present. Strontium may be introduced from without, but its presence is not hurtful. Among poisons we may have lead, antimony, arsenic, mercury, and copper; or perhaps tin, thallium, iridium, silver, bismuth, nickel, and zinc.

The method of obtaining the bright-line spectra of the alkalies and alkaline earths was referred to in the second chapter, to detect them in a tumour or in a calculus, or in the residue of any physiological or pathological fluid, it is necessary to reduce these to a white ash in a platinum or porcelain crucible, then wash the ash with water, with hydrochloric acid, and finally with nitric acid. A piece of platinum foil is then dipped in this solution and held in the Bunsen flame, when the bright lines of the alkalies and alkaline earths appear if the latter are present. Mr Lockyer describes

* It is hardly necessary to explain that by the above statement I do not mean that the metals are present as such, but in the form of salts.

in his book, 'Studies in Spectrum Analysis,' a method of observing the spectra of salts in solution, by means of a small steam spray apparatus, such as is used for the throat, which blows the spray of the solution into the Bunsen flame.

The ash of the human body dissolved in hydrochloric acid gives, when its spectrum is examined, several bright lines, shown in Chart III, Sp. 24. According to Thudichum, the lines are those of six metals—potassium, sodium, lithium, rubidium, cæsium, and calcium. The wave-lengths corresponding to the bright lines of these metals will be found in Watts's 'Index of Spectra.'* Amongst the poisonous metals thallium and iridium are the only two whose spectra can be studied by means of the Bunsen flame.

The spectra of some of the poisonous metals.—The other poisonous metals require the heat of the electric spark, from a battery and an induction coil, to be sent through platinum electrodes coated with the metal (see Chap. II). The most important of the poisonous metals, and the only ones which will be referred to here, are antimony, arsenic, mercury, copper, and lead. These metals are easily separated in the metallic state from solutions which contain their salts by means of electricity (electro-deposition).

Dr Emerson Reynolds † thus describes the method of getting the deposit of the metal on the platinum and the subsequent proceedings: "The plan I prefer

* As almost all works on chemistry contain charts of the bright-line spectra of the alkalis and alkaline earths I have not given one in this volume.

† See Appendix.



for obtaining the deposit of poisonous metal on the platinum is applicable to all the cases just mentioned, and is as follows:—A neat and small glass funnel, having a rather fine stem, is procured, and fixed securely in the usual position for filtration by means of a wooden clip grasping the stem. A bundle of four or six short platinum wires, each about as thick as an ordinary sewing needle, and half an inch long, is now to be bound together by means of a twist or two of very fine platinum wire, one end of the latter being left free so as to facilitate connection of the bundle with a galvanic cell. The bundle is then passed from below up into the stem of the funnel, until half the wire is within the tube; it is then secured in position by means of a little clean cotton wool. When the funnel is filled with water, the wire plug should only allow the liquid to flow out in drops, succeeding each other with moderate rapidity. A long platinum wire is made to dip into the funnel, and to reach down to within half an inch of the top of the wire secured in the lower portion of the stem. It is obvious that when a solution of an easily reducible metal is placed in the funnel, the wire plug connected with the zinc end of a small battery, and the upper wire with the opposite pole, the metal contained in the liquid will deposit on the platinum as it filters through the wire bundle. The whole of the liquid can in this way be certainly brought into contact with the pole on which it is disposed to deposit, and a very small amount of poisonous substance separated from a bulky solution.

“On carrying out this simple but effective plan, it

is necessary that the liquid should be decidedly acid, preferably with nitric acid, in order to avoid the possible production of gaseous arseniuretted hydrogen should arsenic happen to be present. The liquid ought also to be free from suspended matter, in order that the wire filter may not be stopped through ordinary filtration of the liquid. Finally, the battery power required is very trifling, a single Groves's cell being quite sufficient.

“Having obtained the metallic deposit on the wire, the bundle is next removed, and the coated ends of two of the wires placed opposite to each other, within striking distance, in a convenient holder, and the induction spark passed between them, the light being then observed with the spectroscope. If a pair of pure platinum wires be fitted in another holder, and also placed in the path of the current, so that a spark may pass between their points also, the spectrum of the light from this second source may be compared with that from the first, by reflecting the rays into the instrument by means of the little prism placed in front of the slit.” The position of the lines is then measured on the scale, and these measurements reduced to wave-lengths by the curve, described in Chapter II. The most useful wave-lengths for the identification of these metals are as follows :

Arsenic.—The best lines by which to identify this metal, are two strong ones, on the more refrangible side of the sodium line. Thalen's wave-lengths for these lines are, commencing from the red side of the spectrum—6169, 6110, 5651, 5558, 5498, and 5332.

Antimony.—The most important line is that having the wave-length 4711, in the pale-blue part of the spectrum. Twelve easily observed lines are grouped close to and on the refrangible side of the sodium lines, while there are many others along the spectrum on the opposite side of D. Beginning from the red end, the wave-lengths of the most useful lines are 6301, 6129, 6078, 6003, 5909, 5894, 5638, 5567, 5463, 4948, 4711, and 4352.

Mercury.—A single strong mercury line occurs on the red side of D, and a bright double line a little more refrangible than D. The wave-lengths of the principal lines are 6151, 5789, 5768, 5678, 5460, 5426, and 4358. The last line is a rich blue colour and strongly marked.

Copper.—There are six easily distinguished lines in the copper spectrum, one being less refrangible than D. The wave-lengths are 6380, 5781, 5700, 5217, 5153, 5105, and a number of blue lines.

Lead.—Gives seven well-marked lines distributed over the spectrum. A strong red line, with wave-length, 6656; a green line 5607, then 5546, 5372, 5045, 4386, and 4246, the last two are of a violet colour.*

There are two more instances of the application of the study of bright-line spectra to medicine which will probably be turned to good account in the future; the first is :

The detection of morbid gases by means of their spectra.—Gases are examined in Geissler's tubes by

* The bright-line spectra of the other metals will be found described in most treatises on the spectroscope, but it would be beyond the scope of this book to go further into the subject here.

passing an induction spark through them, and morbid gases can be examined in the same manner. The tube is filled with the gas, and then exhausted with the air-pump, until the pressure within it is only $\frac{1}{600}$ to $\frac{1}{700}$ part of the pressure of the atmosphere. The spark is then passed. The gas becomes hot and luminous, emitting lights of different colours, and giving, when examined with the spectroscope, a spectrum different for each gas examined. Thudichum remarks, "It must be left to the future to decide how far these spectra can be practically utilised in the diagnosis of gases which occur in the animal economy. Such gases as occur in morbid emphysemata (in the cellular tissues of cows during cattle plague, in contused limbs with effused blood) in the blood in various conditions, in certain internal cavities or tissues as results of disease, have hitherto mostly escaped analysis, as their small quantity makes the ordinary methods inapplicable. The attention of inquirers must henceforth be directed upon their analysis by means of the electro-spectroscopical method."

The Chemical Circulation.—*Secondly, the spectroscope may be used to determine the time that certain salts take to reach any part of the body.*—Dr Bence Jones used it for this purpose. He imagined "that there are good grounds for believing that there exists within us, in addition to the mechanical or animal circulation of the blood, another and a greater, and a more strictly chemical circulation, closely resembling, if not identical with, that which obtains in the

lower divisions of animals and in vegetables. A circulation in which substances continually pass from the outside of the body into the blood, and through the blood into the textures, and from the textures either into the ducts, by which they again pass back into the blood, or are thrown out of the body, or into the absorbents, by which they are again taken back into the blood, again to pass from it into the textures." He calls this "the chemical circulation," and his experiments were made in order to determine "where diffusing substances go to, how long they are in going out of the stomach into the textures, how long they stay in the textures, and how quickly they cease to appear in the excretions." With the assistance of Dr Dupré, he made use of the spectroscope to answer these questions. He first shows how the spectroscope can detect of—

Chlorate of soda . . .	$\frac{1}{195}$	millionth of a grain
Carbonate of lithia . .	$\frac{1}{8}$	„ „
Chloride of strontium . .	1	„ „
Chloride of barium . .	1	„ „
Chlorate of potass. . .	$\frac{1}{65}$	thousandth of a grain
Chloride of lithium . .	$\frac{1}{12}$	millionth of a grain
Chloride of rubidium . .	$\frac{1}{16}$	thousandth of a grain
Chloride of cæsium . .	$\frac{1}{25}$	„ „

They (Jones and Dupré) then searched for lithia in many vegetable and animal substances, and found it in the following :

“ In Potatoes—seldom.	In Tea—slight traces.
Apples—sometimes.	Coffee—slight traces.
Bread—traces.	Ale—slight traces.
Cabbage—distinctly.	Porter—slight traces.
Rhine wines—always.	Mutton—none.
French wines—distinctly.	Beef—none.
Sherry—distinctly.	Milk—none.
Port—distinctly.	

“ It had already been found—

“ In Sea-water.

Kelp.

Spring-water sometimes.

Ashes of wood grown in the Odenwald.

Russian and other potashes.

Tobacco.

Vine leaves and grapes.

Ashes of the produce of the fields in the
Palatinate.

Milk of animals eating the produce.

Ash of human blood and muscle.

Meteoric stones.

All the drinking waters of London, &c.

“ The spectrum of lithium is very characteristic and very perceptible, and some approximation to a quantitative determination may be arrived at by observing the amount of substance that requires to be burnt to obtain the reaction ; and by the necessity, in some cases, for the removal of interfering substances previous to the combustion. Thus three degrees may readily be

observed. The highest amount of lithia is present when each particle of the substance introduced into the flame gives the lithia reaction ; and a smaller amount of lithia is present when the whole of a lens or of an organ must be extracted with water, to remove the lithia previous to the combustion ; and the smallest trace is present when the substance has to be incinerated, the ash treated with sulphuric acid, the excess of acid driven off, the dry residue extracted with absolute alcohol, the alcohol evaporated, and the dry residue tested. These three quantities may be designated as the slightest trace, a trace, and plenty.

“ As soon as experiments on man and animals showed that the infinitesimal quantities taken in with the food were rarely to be perceived in the textures, experiments were made to determine how quickly the lithium diffused from the stomach into the blood circulation, and from the circulation into the textures, and whether it was to be found in those distant parts of the textures where no circulation existed, and especially in the lens of the eye.

“ The following table gives the experiments made on the rate of the passage of chloride of lithium from the stomach, not only into the circulation, but out of the circulation into the textures of a guinea-pig :

After $1\frac{1}{2}$ grs. were taken.		In 3 days plenty was found everywhere.	
3	„	15 minutes	everywhere except in the lens.
3	„	30	„ „ „

After 3 grs. were taken. In 30 minutes everywhere, and			
			traces in the lens.
3	„	30	„ everywhere, and
			outer part of the lens.
3	„	60	„ „ „
3	„	60	„ everywhere ex-
			cept in the lens.
3	„	2 $\frac{1}{4}$ hours	everywhere, and
			throughout the lens.
3	„	4	„ „ „
3	„	8	„ „ „
3	„	24	„ „ „
3	„	26	„ „ „
$\frac{1}{4}$	„	5 $\frac{1}{4}$	„ everywhere except
			in the lens.

“ It follows from these experiments that three grains of chloride of lithium given on an empty stomach, may diffuse into the cartilage of the hip-joint, and into the aqueous humour of the eye in a quarter of an hour. In very young and very small pigs, the same quantity of lithium may in thirty or thirty-two minutes be found in the lens of the eye, but in an old pig in this time the lithium will have got no farther than the humours of the eye. If the stomach was empty when the chloride of lithium was taken, then in one hour the lithium may be very evident in the outer part of the lens, and very faintly in the inner part; but if the stomach be full of food, the lithium does not in an hour reach the lens. Even in two hours and a half the lithium may be more marked in the outer than in the inner part of the lens.

In four hours the lithium will be in every part of the lens, but it will still be more evident in the humours than in the lens. Even in eight hours the centre of the lens may show less than the outer part. The lithium will be found in as great quantity in the centre of the lens as in the outside after twenty-six hours." When the lithium was injected "into the skin" instead of being put in by the stomach, "three grains of the chloride showed in twenty-four minutes lithium in the lens and every texture; in ten minutes, slightly in the lens but plenty everywhere else; in four minutes no lithium was in the lens, but plenty in the aqueous humour of the eye and in the bile; one and a half grain in five minutes showed no lithium in the lens, but plenty in the aqueous humour and in the bile."

It was thus shown "that lithium will pass everywhere into the textures in between four and fifteen minutes, when injected into the circulation, and between fifteen minutes and twenty-six hours when taken in by the stomach. Some experiments were afterwards made to determine after how many days the lithium ceased to be detected in the textures after it had been taken. Usually three pigs were taken: to one no lithium was given, the second was killed in a few hours after a dose of lithium, and the third was given the same dose and killed after many days."

"The following table shows the rate at which chloride of lithium passes out of the textures:

2 grs. in 6 hours gave plenty everywhere. In 6 days gave no trace in the alcoholic extract of the kidneys, livers, or lenses.

2 grs. in 6 hours gave plenty everywhere, and in 6 days result as before.

2 grs. in 6 hours gave plenty everywhere, and in 4 days gave none in the lens.

1 gr. in $5\frac{1}{2}$ hours showed partly in the lens. In 3 days gave faint traces in the lens.

“It follows from these and other experiments that twice in six days, and once in four days, two grains of chloride of lithium, which in six hours gave lithium everywhere, in six days ceased to be detectable in the lens, and that even in three days the lithium is most probably diminishing in the lens.” Dr Bence Jones then, by the help of Mr Bowman and Mr Critchett, endeavoured to trace the passage of lithium “into that part of the body which is most distant from the blood circulation in man.” Twenty grains of carbonate of lithium dissolved in water were given in a few minutes, or a few hours, or a few days before the extraction of a lens affected by cataract. (Some lenses affected with the same disease having been previously examined so as to determine whether lithia might not have been naturally present, and in only one instance was the faintest trace discovered.)

These experiments taught “that in the human body twenty grains of carbonate of lithia taken into the stomach in two and a half hours will have partly passed into every particle of the textures and beyond the blood circulation even into the most distant parts, and in three and a half hours it will be distinctly present in each particle of the lens.”

“After four days it will still be distinctly present in each particle of the lens.

“After five days it will have begun most clearly to pass out of the lens, and in seven days scarcely the smallest trace will be detectable there.

“A long series of experiments on the passage of lithium out by the excretions, after it had been taken in by the mouth, showed nearly the same fact, namely, that after a dose of twenty grains, the lithium was not entirely thrown out of the body under six, seven, or eight days.

“Thus, then, both in animals and man the same law obtains. A single dose of lithium in a few minutes passes through the circulation into all the ducts, and into every particle of the body, and even into the parts most distant from the blood circulation. There it remains for a much longer time than it took to get into the textures, probably for three or four days, varying with the amount taken ; then it diminishes, and finally, in six, seven, or eight days, the whole quantity is thrown out of the body.”

Chloride of rubidium and cæsium are also proved to follow the same law as lithium, in short, these substances were found to pass into the lens, being detected there ; but twenty grains of chloride of rubidium or chloride of cæsium had to be given to guinea-pigs before the lenses of the animals gave the spectrum reaction of these metals.

I have given Dr Bence Jones's own words in most of the above extracts from his book, his experiments

and deductions being in my opinion too interesting to allow of their being abridged.

The spectroscope can also be used to detect the presence of various metals in mineral waters.

I would refer the reader to Roscoe's 'Lectures on Spectrum Analysis' for an account of the discovery of cæsium and rubidium in the waters of Dürkheim by Bunsen, &c.

CHAPTER IV.

PHYSIOLOGICAL ABSORPTION SPECTRA.

THE study of the absorption spectra of various physiological fluids has taught us more than their ordinary chemical analyses. More especially is this the case with blood; for not only has the spectro-scope shown why arterial differs from venous blood in colour, but it also enables us to detect the minutest trace of blood; and also to understand how certain gases, &c., of a poisonous nature put an end to animal life. The study of the action of various reagents upon blood out of the body has explained the action of reagents within the body. For just as acids and alkalies, respectively, split hæmoglobin up into acid and alkaline hæmatin in the laboratory of the chemist, so also is hæmoglobin split up by the same or similar agents in some pathological fluids within the body. As an instance, the hæmatin which occurs in ovarian and parovarian cysts may be mentioned, which I shall describe for the first time further on.

An examination of the bile of various animals has led me to believe that the so-called urobilin is present

in the bile of many of the lower animals,* and it may be said to be present in the bile of almost all animals that possess a receptacle for this fluid. This colouring matter is constantly present in human urine, and is abundantly present in the bile of the mouse, which gives its absorption band with great distinctness. It was known to be capable of production in the laboratory by the action of acids on bile, and, as will be described further on, Maly has produced it from another bilepigment; but I can affirm that it is constantly present in all kinds of bile, and in most of them its spectrum can be seen without any treatment whatever.

Before proceeding to the description of various physiological and pathological absorption spectra, it will be necessary to give an account of some recent researches on absorption spectra in general, which are of great importance, as they teach us the necessity of caution in drawing inferences as to identity in chemical composition between two bodies, from the fact that solutions of these bodies give bands in the same part of the spectrum. It would at first sight appear that since identity of spectrum does not ensure chemical identity, the study of absorption spectra is entirely useless, because it is upon this principle that the whole of spectrum analysis, as applied to the study of the chemical elements, is based; but a little consideration will show that the value of absorption spectra, as tests for the presence of pigmented substances, is not diminished by these discoveries.

Vogel's researches on absorption spectra.—Experi-

* *i. e.*, without any treatment whatever.

ments undertaken by Herr Kundt had shown that the stronger the dispersion of the solution, the nearer to the red end will the absorption band given by the substance in solution be found. In Kundt's experiments he overlooked the fact that, when changing the solvent, the whole character of the spectrum was changed, so that comparison of one solution with another was rendered difficult.

Herr Vogel has investigated the different effects produced upon the position of absorption bands in the spectrum by the nature of the medium (in which the body giving these absorption bands is dissolved); he was induced to undertake this research through having observed remarkable differences between the spectra of solids and of solutions of the same solids. In his experiments, instruments of small dispersion were used, because, as mentioned before, such instruments are more suitable for observing absorption spectra, owing to the bands being better defined, and since the whole spectrum can be observed at the same time. "The absorption spectra of solid salts and pigments were obtained from thin layers of these substances, prepared upon glass plates, through evaporation of a few drops of solution." Vogel shows not only *that differences occurred in the spectra of one and the same solid substance and its solution*, but often a *striking coincidence in the position of the absorption bands belonging to totally different substances (e. g. nitrate of uranium and potassium permanganate)*.

The vapours of iodine, hyponitric acid, indigo, &c., had their spectra examined, and these were compared,

in most cases, with the aqueous, alcoholic, and other solutions of the same substances.

The results arrived at may be thus summed up :

1. Considerable differences exist between the spectrum which a substance gives in the solid, liquid, or dissolved and gaseous state. Characteristic bands which are shown in the spectrum of one state, are either not reproduced in that of the others (this is the case with chrome alum, chloride of cobalt, iodine, bromine, naphthaline red, fuchsine, indigo, cyanine, aniline blue, methyl violet, eosine, carmine, purpurine, alizarine, santaline), or they reappear in a different position, or of different intensity (*e.g.* nitrate of uranium, permanganate of potassium, hyponitric acid, alcanna red). Sulphate of copper and chlorophyll show the same absorption both in the dissolved and in the solid state.

2. The spectra given by the same substance when dissolved in different media are the same in some cases (purpurine in alcohol or sulphide of carbon, aldehyde green in water or alcohol, methyl violet and indigo-sulphuric acid in water or amylic alcohol); in other cases they differ only in the position of bands (chloride of cobalt, fuchsine, coralline, eosine, and iodine green, in aqueous or alcoholic solutions); and again in others, their character is totally different, so that no point of coincidence remains (iodine in sulphide of carbon or alcohol, naphthaline, aniline blue, purpurine, hæmatoxylin, brasiline, in water or alcohol).

3. The rule established by Kundt, viz. that the absorption bands of a body in solution lie the nearer towards the red end of the spectrum the greater the dis-

persion of the dissolving medium is, has not been confirmed in many cases; on the contrary, in some instances the absorption bands move towards the blue in a solution of greater dispersion (nitrate of uranium and blue chloride of cobalt in water and alcohol); in other cases their position remains unaltered for various media (hyponitric acid in air and benzol, indigo-sulphuric acid and methyl violet in water and amylic alcohol, aldehyde green in water and alcohol, purpurine in sulphide of carbon and alcohol). In some cases a great difference in the sense of Kundt's rule becomes apparent, while in others for the same spectral region but a very trifling one appears, according to the nature of the pigment (coralline and fuchsine). Indeed, it happens sometimes that certain bands are in the same position with different dissolving media, while others which are *simultaneously* visible are displaced (nitrate of uranium in water and alcohol, oxide of cobalt in glass and in water, proto-nitrate of uranium in neutral solution and in a solution of oxalic acid, chlorophyll in alcohol and ether).

4. The position of absorption bands in the spectra of solid and dissolved bodies may be only exceptionally deemed characteristic for any certain body. Totally different bodies show absorption bands in exactly the same position (solid nitrate of uranium and potassium permanganate in the blue; naphthaline red, and coralline in the yellow; indigo, aniline blue, and cyanine in the orange; aldehyde green and malachite green in the orange). Closely related substances sometimes show remarkable differences in the position of their bands

under perfectly equal conditions (solid uranium salts).

5. The rule set up for absorption spectra, "each body has its own spectrum," can be admitted only with great restrictions. The great number of polychromatic substances show different colours and different spectra in the solid state, according to the direction in which they are observed. Most other bodies show different spectra in the solid state from those of their solutions; and in the latter case again, different ones according to the dissolving medium, and the question arises which of all these spectra is the body's *own* spectrum.

These conclusions arrived at by Herr Vogel would appear to diminish the value of bands, but we have another method to fall back on besides observing the mere position of bands, that is, the *changes in the spectra of the same body, caused by various solvents and reagents*. (Thus cyanine and aniline blue dissolved in alcohol give a very similar spectrum, but in water a totally different one. The two bands of O- hæmoglobin are replaced by one when reducing agents are used; those of carmine—which resembles blood in its bands—are not changed, and so on.)

The recognition of a body becomes more certain if its spectrum consists of *several* absorption bands, but even the coincidence of these bands with those of another body, is not sufficient to enable us to infer chemical identity; what enables us to do so with certainty is the fact:—*that the two solutions give bands of equal intensities in the same parts of the spectrum which*

*undergo analogous changes on the addition of the same reagent.**

Fortunately for medical spectroscopy we always use reagents in determining the nature of a substance present in solution; thus, in determining whether hæmoglobin or hæmatin be present, we always add to the solution supposed to contain them, either Stokes' fluid or ammonium sulphide. So that Vogel's researches do not interfere with the conclusions drawn in this book. But they teach us to accept with reserve conclusions hastily drawn from the mere position of bands in a spectrum, unsupported as they often are by more precise analysis. There is no doubt at the same time that Vogel's conclusions are not true for all pigments, the aqueous, the alcoholic, chloroformic, and etherial solution of some substances, do not, at least in the case of some physiological pigments, show such striking differences as the above would lead us to expect; and, as a general rule, two bodies, giving exactly the same spectrum, will be found to have a close chemical relationship, if not an identical composition.† Nor does the colour of the solution always make such a difference to the position of bands, for often fluids of a different colour give the same spectrum, *e.g.*, the bile of the ox and sheep, when fresh and when they are beginning to decompose.

Mixed colouring matters.—Mr Sorby has written an excellent paper on the "Examination of mixed Colouring Matters by the Microspectroscope," from which I

* 'Nature,' vol. xix, p. 495.

† See remarks on wave-lengths, Appendix I.

quote a few extracts, as his remarks will be found exceedingly useful by those who wish to understand the methods which it is necessary to adopt before drawing conclusions from the position of bands in a spectrum. The paper will be found in the 'Monthly Microscopical Journal' for 1871 (vol. vi, p. 124, *et seq.*). He says :—"In studying the colouring matters, soluble in water, that may be obtained from various kinds of algæ, for which special names have been proposed, as though they were single and simple substances, I have been led to conclude that they are in some cases mixtures of at least four, which are readily distinguished by their spectra. The facts which have thus presented themselves, have so impressed me with their importance in such inquiries, that I think it may be well to make the study of mixed colouring matters the subject of a special communication."

"The manner in which the mixed nature of some colouring matters may be ascertained from their spectra, has been already described by Professor Stokes and others, as well as in previous papers by myself; but in order to make this communication complete in itself, I must be allowed to again describe some of them. I shall not attempt to enter into the chemical part of the subject, or to treat of the separation of different substances by purely chemical methods, such as the solubility or insolubility in various reagents, but confine myself almost entirely to those processes in which the examination of the spectra is of primary importance. I scarcely need say that the coloured material should be separated, as far as

can be conveniently managed, into that which is soluble or insoluble in such simple solvents as water or alcohol; but at the same time there are cases in which such a difference in solubility does not appear sufficient to prove that the colouring matter itself differs essentially. The spectra seem to show that occasionally the presence of some other substance, insoluble in water, which has a strong affinity for the colouring matter, is the true cause of their variations. I shall, therefore, presume that we have to deal with colouring matters, separated from any others that differ materially in their solubility.

“There are few cases in which the mixed nature of a coloured aqueous solution can be more easily ascertained, than when the constituents differ so much in character, that the addition of some reagent will more or less completely destroy the spectrum of one, without having any effect on that of the other. For this purpose no substance is superior to sulphite of soda. Without producing any real decomposition, this almost entirely removes the detached absorption at the red end of the spectrum of certain colours, but has no effect whatever on that of the others. In the case of some colours it thus acts when the solution contains excess of ammonia, but in the case of others it has then little or no action, but removes the absorption when the solution contains excess of such a moderately weak acid as citric.” Mr Sorby then goes on to show how this method is applied in the case of the colouring matter of certain plants. He then shows how we may separate two colouring matters from each other by

ether, when the ethereal solution rises to the top, and the aqueous falls to the bottom. "It will be thus seen that, if a mixture were thus treated, a partial separation might often be effected, and on evaporating to dryness, redissolving in water, and comparing the spectra, either in the natural state or after reagents had been added, the differences might clearly prove that two or more colouring matters were present." . .

"When a solution contains more than two colouring matters the recognition of each becomes somewhat more difficult; but still, by following out this system, and dividing the material into more than two portions, a very good opinion may be often formed of the general optical properties of each substance. When some of them give well-marked and characteristic absorption bands, and when the absorption of others may be removed by the addition of sulphite of soda, the study of a complex mixture is very greatly facilitated, and especially if the spectrum of one or more of the constituents is of such a marked character, that it can be at once recognised as that of some substance already known in a pure state. A tolerably good opinion may then be formed of the spectrum of the rest by, as it were, subtracting that of the known constituent. This leads me to the description of the spectra of certain colouring matters, which are met with so far separated naturally, that their compound characters may be inferred without reasonable doubt, and confirmed by a more extended examination."

"I have lately found that many interesting facts may be observed, by examining the spectra of sub-

stances in their natural state without extracting the colouring matter. Frequently they are so opaque that it is requisite to use a very bright light to penetrate through them."

The consideration of various absorption spectra which are described in his paper led Mr Sorby to draw these conclusions :

1. "When a spectrum shows two absorption bands, they should not be considered due to one single substance, until satisfactory evidence of the fact has been obtained. The solution should be allowed to undergo slow decomposition, and be repeatedly examined, in order to ascertain whether both bands disappear in the same proportion, and also the reaction of various reagents observed, in order to learn whether one band can be permanently removed without the other, making, of course, due allowance for any change that may depend merely on an acid or alkaline reaction."

2. "When more than two bands are seen in the spectrum, and they are not at nearly equal intervals, the compound nature of the substance may be considered so probable that further examination should certainly not be neglected."

3. "When there is broad shading about a narrow absorption band it is important to ascertain whether or not it is due to the same substance. There are certainly many cases in which I have always concluded that both are due to the same," but examples show that there are exceptions to this rule.

"The occurrence of so many associated colouring matters, as in algæ, may be rare. It must not be

supposed that I imagine whenever there are two or more absorption bands they are due to two or more independent substances. As an example of what I look upon as satisfactory proof of the contrary, I will describe some facts connected with the well-known spectrum of blood. If after exposure in a dry state to the air for some weeks, until the hæmoglobin has been converted into methæmoglobin, a small quantity of the double tartrate of potash and soda be added to the aqueous solution, and afterwards a very minute portion of the double sulphate of protoxide of iron and ammonia, the methæmoglobin is deoxidised and reconverted into hæmoglobin, as described in my late paper 'On Blood Stains' (*vide* Appendix). Here, then, we have a decomposition gradually effected by the atmosphere, and if two different substances had been present it is extremely probable that they would have varied in the rate of change, so that there would finally have been an alteration in their relative proportion, and thus, when deoxidised, there would not have been the same relation between the absorption bands as in fresh blood. I find, however, that the agreement is complete. Moreover, if the colouring matter had been a mixture of two substances, it is extremely probable that there would have been some such variation in their relative amount in the blood of very different animals, as occurs in the colouring matters of various algæ. In order to ascertain whether this is the case, I carefully compared side by side the spectra of human blood and that of the small annelids so common in stagnant pools, and found that the position and

relative intensity of the two bands were exactly the same."

His conclusions as to relationship between pigments which give spectra like each other, and the light which the study of wave-lengths has thrown upon this subject, will be noticed after the absorption spectra of the blood, bile, and urine, &c., have been described.

Methods of observation.—The general method of observing the absorption spectra of fluids was described before (see Chapter II). For most purposes, when there is abundance of material, the chemical spectroscope is the most satisfactory instrument for working at absorption spectra, because the solutions being placed in test-tubes we are the better able to study the action of reagents; and when, as in examining the spectrum of Gmelin's test, there are strata of different densities, we can examine each separate stratum with great satisfaction. But when the fluid is small in amount, or when we want to get a spectrum with the light reflected from the surface of the body, or to examine blow-pipe beads, &c., the microspectroscope is to be preferred; moreover, the definition of the latter is much better than the former, for we can often see with the microspectroscope faint absorption bands which escape notice when examined with the chemical spectroscope. Different depths of fluids are also examined more easily with the microspectroscope, as we can increase or diminish the depth of fluid in a cell, in a much shorter space of time than it takes to bring a succession of different sized test-tubes before the slit of the chemical spectroscope.

For detecting blood in urine, a small pocket spectro-

scope such as that shown here, will be found very convenient, and considering the size of the instrument, it is astonishing what an amount of work can be done with it. For those who can afford it, two spectroscopes will be found to give more satisfactory results than one, but if the additional cost is an object, the Sorby-Browning microspectroscope with the photographed scale is amply sufficient for all purposes.

FIG. 13.



The spectrum of oxidized hæmoglobin.—The blood bands.—Blood, as is now known to every reader of physiology, owes its colour and its spectrum to hæmoglobin; that this is the case, is proved by the fact, that a solution of blood gives the same spectrum as hæmoglobin, when the latter has been separated from the former either in the crystallised or amorphous condition. The defibrinated blood of the dog or pig, or any other vertebrate animal, is amply sufficient for the study of its optical characters, or a drop of blood from the finger can be made to show the spectra of oxidized and deoxidized hæmoglobin, of alkaline and acid hæmatin, and of deoxidized hæmatin. Of course if we wish to study the characters of chemically pure solutions, we must separate the hæmoglobin by one of the usual

methods.* Having obtained some blood, and having made a solution with water, we can see by putting it before the slit of the chemical spectroscope, or in a cell beneath the microspectroscope, the spectrum of hæmoglobin in the oxygenated condition. If the solution be too strong all light will be stopped, but if it is diluted sufficiently, a little of the red and the orange appear; diluted still more, a little green appears; but between the orange and the green there is a broad, very dark band. If we compare its position with that of the Fraunhofer lines we find that it extends from beyond D on the red side, to near b on the violet side (Chart 1, 2). On diluting still more, this band is found to be composed of two, one of which, next D, is darker and more strongly marked than the other, while the latter is broader and more washed out at the edges. By further dilution we narrow these bands, and we can go on diluting until the band next the violet end of the spectrum disappears, and that nearest D only is left. By diluting still more the latter band also disappears, and nothing but the continuous spectrum of the light-source is left.†

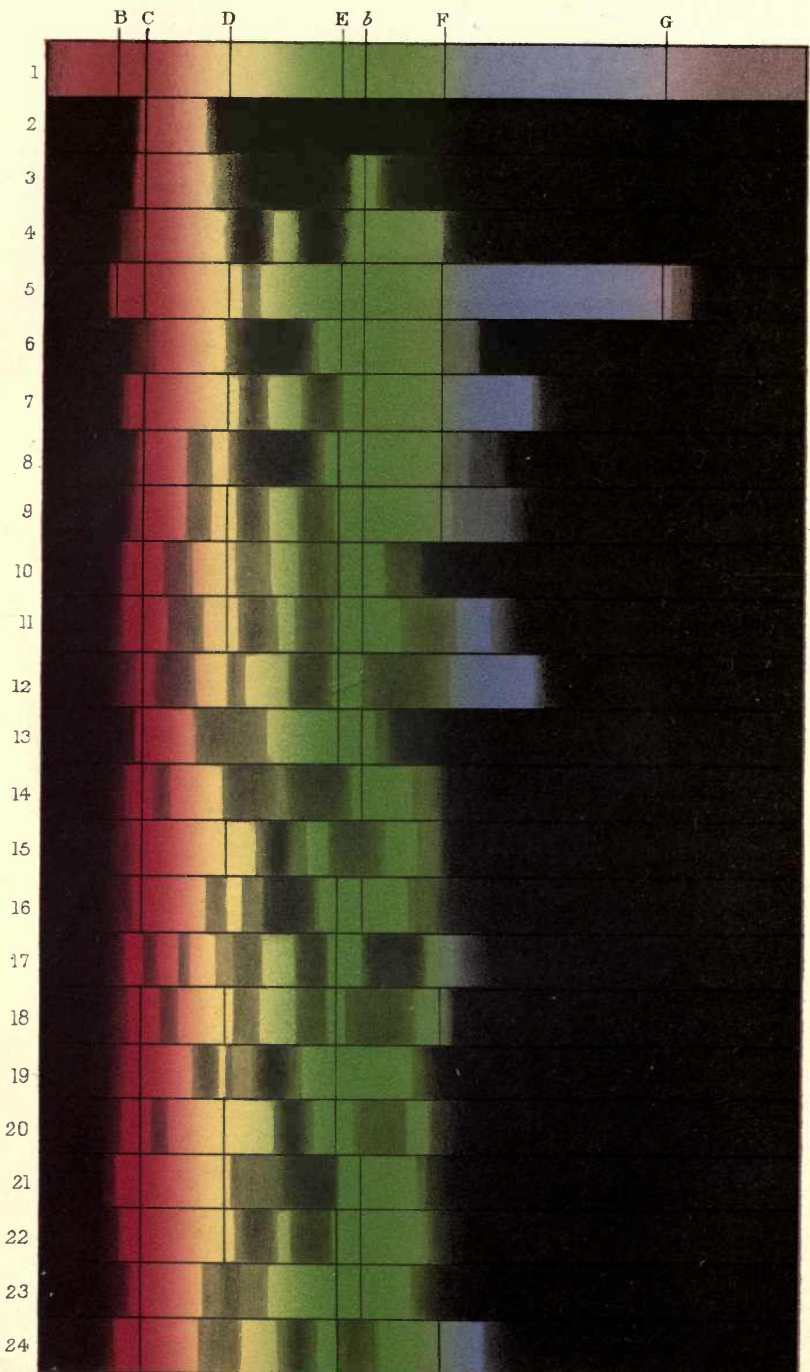
By examining in the cell of a microspectroscope a solution sufficiently strong to allow only the red to come through, and then gradually diminishing the thickness of the layer, the same appearances as those got by gradual dilution can be obtained. We can get

* See Thudichum's 'Chemical Physiology,' or any good treatise on physiological chemistry.

† These two bands are those of oxidized hæmoglobin, the "scarlet cruorine" of Stokes, the "oxidized hematocrystalline" of Thudichum, and the appearances described above are shown in Chart I, 2 to 5.



CHART I.



Cha^s A. MacMunn ad nat. del.

Hanbary lith.

the same two bands by examining the frog's web with the microspectroscope, by reflecting light from the surface of human skin into the microspectroscope, or by holding the ear of a rabbit between the light and the slit of a spectroscope; the red fluid of the earth-worm and of the house-fly yields the same spectrum. So delicate is the spectroscopic test for blood that we can detect by means of the microspectroscope as little as the $\frac{1}{1000}$ th of a grain of hæmoglobin.

Distribution of hæmoglobin in the Animal Kingdom.

—Hæmoglobin has been detected by means of the spectroscope:—(1) In the blood of vertebrates located in the red corpuscles, with the exception of the *Amphioxus*, in which it is found in the plasma, not in the corpuscles. (2) In most of the striped muscles of mammals and birds; but only in the cardiac muscles, and in certain very active muscles of other vertebrates. (3) In the unstriped muscle of the human rectum; in other unstriped muscles it is usually absent. (4) Its presence is variable in the *Annelidæ*, in some of which it is accompanied by another dichroic substance very like hæmoglobin in its spectroscopic relations. (5) It is present in the fluid from the perivisceral cavity of the leech. (6) It is distributed through the plasma of the so-called blood of the larva of *Chironomus*, but it has been sought for in vain in other insects, myriapods, and arachnids. (7) In the blood-plasma of certain crustaceans, while it is absent in others. (8) It is absent, for the most part, from the blood of molluscs, though it is present in the blood of a gasteropod (*Planorbis*). (9) It is met with in the

muscular fibres from the pharynx of gasteropod molluscs, as *Limnæus* and *Paludina*, although it is wanting in their blood. Lankester has observed that among gasteropods it is those muscles which are most active and most powerful that are furnished with hæmoglobin. This author also describes chlorocruorin from the green blood fluid of *Sabella*; it was found to give two absorption bands, not in the same position as those of oxidized hæmoglobin, but which, on the addition of reducing agents, were changed into one. He also showed that chlorocruorin and hæmoglobin have a common base in "*cyano-sulphæm*,"* and perhaps in Stokes' reduced hæmatin, of which a description will be given afterwards.

Preyer's method of calculating the percentage of hæmoglobin by means of the spectroscope.—Before describing the spectrum method of estimating the amount of hæmoglobin in blood, it may be mentioned that Preyer had arrived in 1869 at the following conclusions in regard to the effect which solutions of hæmoglobin of different strengths, when examined in a layer one centimètre deep, had upon the spectrum; these numbers will be found very useful:—

(a) A solution containing from 0·003 to 0·009 parts per cent. showed very faintly one band.

(b) A solution containing 0·01 per cent. gave two bands very feebly.

(c) A solution containing 0·09 per cent. showed a difference of intensity in the shading of the bands.

(d) A solution of 0·8 per cent. gave only one broad

* See Appendix for title of Prof. Lankester's paper.

band, both bands having coalesced; in addition to the red from a, to near D, there was only a green stripe observable between b, and F, but near b. This green stripe is not seen if the solution contains 0·9 per cent., but is distinctly seen if the solution contains 0·7 per cent. of hæmoglobin. Solutions containing more than 7·3 per cent. of hæmoglobin allow no light at all to pass.

The solution (*d*) containing 0·8 per cent. of hæmoglobin is taken as the normal solution for comparison in determining the percentage of hæmoglobin in the blood.

To estimate the amount of hæmoglobin in a specimen of blood, we must make a solution of a measured or weighed quantity of blood in water, and then find, with the aid of the spectroscope, what degree of dilution is necessary to bring it to such a strength that only the red rays are transmitted. The point of dilution at which green is extinguished has been found by Preyer to be very constant (the solution containing 0·8 per cent., one centimètre in thickness, see *above*), and is therefore used as the standard fluid for comparison. To make the standard solution, we introduce a concentrated solution of a known weight of pure hæmoglobin crystals into a glass chamber (hæmatinometer) of which the parallel sides are one centimètre apart from each other. The chamber is then placed in front of the spectroscope, the source of light being a paraffin lamp. Distilled water is then gradually added from a divided burette so long as all of the spectrum is extinguished except the red. The moment the green begins to appear the operation is ended.

The volume of the diluted solution is determined, and the exact conditions, viz. the distance of the lamp and the glass chamber, and the width of the slit are noted down. The percentage of hæmoglobin in the solution is that at which, *under the given conditions*, complete absorption of the green takes place. It may be called k .

In order to determine the percentage of hæmoglobin in any given specimen of blood, all that is required is to repeat the operation just described with the blood; thus, a small quantity of fresh blood, which has been well agitated with air and defibrinated, is introduced into a finely graduated small pipette, from which exactly one cubic centimètre is delivered into the glass chamber, and diluted before the slit of the spectroscope (the liquid being carefully stirred after each addition of water) until the green begins to appear. At the moment the green is seen the liquid contains a percentage of hæmoglobin equal to k . If the volume of distilled water, including the cubic centimètre originally added, be designated c , and the original volume of blood b , the percentage of hæmoglobin which the blood contains is readily calculated according to the formula :

$$\frac{x}{k} = \frac{b+c}{b};$$

Therefore, as $b = 1$, we have

$$x = k(1 + c).*$$

According to M. Rajewski this method is less exact

* 'Handbook for Physiological Laboratory.' 1873. By Klein, Sanderson, Foster, and Brunton.

than that of *Hoppe-Seyler*. (M. Quincke made a useful modification in this method by making use of a prismatic vessel attached to a graduated scale, in which a 10 per cent. solution of blood was introduced.)

Among the other methods of estimating hæmoglobin may be mentioned—(1) By the estimation of iron. (2) By the estimation of oxygen. (3) By the estimation of hæmatin. (4) By the method of comparison of Hoppe-Seyler. (5) By J. Worm Müller's method. (6) By the fluid scale of Welcker. (7) By Welcker's scale of blood stains. (8) By the painted scale of M. Hayem. (9) By the globulimeter of Mantegazza. Or (10) By the hæmochromometer of Malassez.* It will be thus seen that there are a great many methods of estimating hæmoglobin besides the spectroscopic one.†

The absorption bands of blood were first described by F. Hoppe‡ in 1862, and his experiments being repeated by Stokes, the results he arrived at were confirmed.

Reduced hæmoglobin.—Professor Stokes§ not only confirmed Hoppe's experiments, but he also found that, by adding certain reducing agents to blood,

* See the 'London Medical Record' for July 15, 1879, p. 256; and title of Malassez's paper, Appendix II.

† Probably we might get a method still more accurate by noting the readings (with the microspectroscope), and calculating the wave-lengths corresponding to, the *edges* of absorption bands in a solution containing a known amount of hæmoglobin, and examined in a layer of the same depth, and comparing with this standard solution, solutions of blood, always diluted to the same amount with water, and examined at the same depth as the standard solution, and noting the wave-lengths of absorption bands given by the latter. I merely throw this out as a suggestion, not having had sufficient time to follow up the idea.

‡ 'Virchow's Archiv, Bd. xxiii, p. 446.

§ Appendix II.

he could change the scarlet blood into purple (or, as he described it), "scarlet cruorine" into "purple cruorine." Supposing that the change in colour arose from reduction he added to blood the fluid which is now called after him, viz. an ammoniacal solution of ferrous sulphate, to which enough tartaric acid had been added to prevent precipitation by alkalies; and he found not only that change of colour took place, but that the spectrum was changed, instead of two bands, only one was seen, having its darkest portion in the position formerly occupied by the space between the blood bands. The fluid he added had a greater affinity for oxygen than had the hæmoglobin, so the latter was robbed of it. Dr M. Foster describes so accurately the spectrum of this solution, that I cannot resist quoting him. He says:—"Examined by the spectroscope, the reduced solution, or solution of reduced hæmoglobin, as we may now call it, offers a spectrum entirely different from that of the unreduced solution. The two absorption bands have disappeared, and in their place is seen a single, much broader, but at the same time much fainter band (see Sp. 6, Chart I), whose middle occupies a position about midway between the two absorption bands of the unreduced solution, though the redward edge of the band shades away rather farther towards the red than does the other edge towards the blue. At the same time the general absorption of the spectrum is different from that of the unreduced solution, less of the blue end is absorbed. Even when the solutions become tolerably concentrated, the bluish-green rays

to the blue side of the single band still pass through. Hence the difference in colour between hæmoglobin which retains the loosely combined oxygen and hæmoglobin which has lost its oxygen and become reduced. In tolerably concentrated solutions, or tolerably thick layers, the former lets through the red and the orange-yellow rays, the latter the red and the bluish-green rays. Accordingly, the one appears scarlet, the other purple.

“In dilute solutions, or in a thin layer, the reduced hæmoglobin lets through so much of the green rays that they preponderate over the red, and the resulting impression is one of green. In the unreduced hæmoglobin or oxy-hæmoglobin, the potent yellow which is blocked out in the reduced hæmoglobin, makes itself felt, so that a very thin layer of hæmoglobin, as in a single corpuscle seen under the microscope, appears yellow rather than red.”

It is exceedingly easy to cause the reduction of hæmoglobin in solution, we can do it by means of indifferent gases, *e. g.* nitrogen, or we can remove the oxygen by means of the air-pump. But the easiest method is by the addition of ammonium sulphide to the solution of blood, or by the addition of Stokes' fluid; the last is not as satisfactory as the ammonium sulphide, since it causes turbidity in fluids where the other does not, and it spoils by being kept; moreover, in using it greater precautions have to be adopted for the exclusion of the air, and it sometimes fails to reduce certain forms of hæmatin, which are at once reduced by ammonium sulphide. If, then, we add to a solution of

blood in a test-tube before the slit a few drops of ammonium sulphide, turn the test-tube upside down once or twice, holding the thumb against its mouth to prevent spilling of the contents, plug it with some cotton wool, pushing the wool down almost to the surface of the fluid, and then gently heat, we notice the red colour of the solution change to purple, and the spectrum presents the appearance so well described by Professor M. Foster, and shown in Chart I, 6. Or, we may add the reducing agent to the solution in a cell beneath the microspectroscope, having taken the precaution to cover the fluid in the cell with a microscopic cover-glass.*

The importance of this discovery of Stokes cannot be over estimated, for not only does it explain the difference in colour between arterial and venous blood, but it also shows us wherein the breathing-power of the red blood-corpuscle resides, and explains phenomena which, before its discovery, were inexplicable.

If, after adding the reducing agent, and after the spectrum has changed as described above, we shake the fluid in the test-tube with air, or stir up the fluid in the cell, the single band again disappears to be replaced by the first observed bands of oxy-hæmoglobin; left to itself for a few moments it again becomes reduced, but can be again changed as before by agitation. In fact, the same result can be made to take place as often as we wish.

* This reduction test distinguishes blood from other pigments, the spectra of which somewhat resemble it, *e.g.* carmine, and turacine, a pigment discovered in the feathers of the Cape lory by Church.

It has been stated repeatedly, that arterial and venous blood owe the difference in their colour to this oxidized and deoxidized condition of the hæmoglobin; but it has been also asserted that venous blood always contains enough oxygen to make it give the spectrum of oxy-hæmoglobin. I shall now show that the latter statement is not absolutely correct. In the case of venous blood after death, there is an exception to the rule.

Spectrum of venous blood after death.—In examining the blood of a still-born foetus with the spectroscope, I thought I had come across an additional test of the viability or rather the non-viability of the foetus, as the blood from the vena cava, right auricle, right ventricle, left auricle, and left ventricle *gave the one band of reduced hæmoglobin*. The blood was examined on a microscopic glass slip, being quickly covered with a cover-glass so that it had not time to become oxygenated. I then examined the blood of adults who had died of various diseases, and in every instance I found that the blood in the right auricle and right ventricle gave the spectrum of reduced hæmoglobin. At the time, I concluded that this reduction was an effect of decomposition, because blood out of the body becomes spontaneously reduced after the lapse of some hours; but a series of experiments on the lower animals has taught me that *hæmoglobin becomes reduced in the act of dying*, provided death is not caused by starvation, cold, &c. (*vide* p. 74). In addition to the method of examination adopted above, I find Husband's capillary vaccine tubes are very useful for the same purpose.

One end of the tube is sealed in the flame, and the tube being then quickly drawn through the flame so as to expel the air, the other end is sealed; in this way we have a partial vacuum in the tube. When blood has to be examined, an opening sufficiently large to allow of the introduction of the tube is made in a blood-vessel, and the former is pushed into it for the distance of half an inch or so; the tube is then held with the forefinger and thumb of the left hand, while its apex within the vessel is broken off, or crushed off, with forceps. The blood rushes into the tube, which is then withdrawn and again sealed in the flame. When this is examined with the microspectroscope, it is necessary to place it on a piece of black paper or platinum foil perforated with a pinhole, so as to stop all surplus light.

The following experiments were made in order to determine within what space of time the reduced hæmoglobin band appears after death.

(1) A rabbit was killed by pithing, and its blood was examined *twenty-seven minutes after death*. The blood, in the right auricle, right ventricle, venæ cavæ, and left iliac vein gave the *reduced hæmoglobin band*; that from the left ventricle and left auricle showed a tendency to reduction; *that in the aorta gave the two-banded spectrum of oxidized hæmoglobin*.

(2) A rabbit was poisoned with strychnine; *ten minutes after death* the blood in the right auricle, right ventricle, and venæ cavæ gave *the band of reduced hæmoglobin*, *that from the left auricle and ventricle and aorta gave the bands of O-hæmoglobin*.

(3) A rabbit was killed by pithing; the blood from the right auricle and ventricle, and *venæ cavæ* gave the band of *reduced hæmoglobin three minutes after death*. Within six minutes the blood in the left auricle and left ventricle showed a tendency to reduction. *The blood in the aorta was not reduced*, and the blood in the ear was not reduced.

(4) A rabbit died from debility; *within half an hour* the blood was examined; that in the right auricle and right ventricle gave the spectrum of *reduced hæmoglobin*; that in the left auricle, left ventricle, and aorta gave the *oxidized hæmoglobin spectrum*.

(5) A hedgehog died from chloroform narcosis. Its blood was examined *eight minutes after death*. It was found to give the spectrum of *reduced hæmoglobin* in the right auricle, right ventricle, and *venæ cavæ*; but that of *oxidized hæmoglobin* in the left auricle, left ventricle, and aorta.

From these and other experiments we may conclude that the hæmoglobin of the blood in the right side of the heart and in the veins is reduced as soon as the animal has ceased to breathe, but that the hæmoglobin of the blood in the left side of the heart and aorta does not become reduced for some time after death, the time varying with the mode of death.

At the time I made these experiments and had drawn these conclusions from them, I was not aware that Professor Hofmann, of Vienna, in the paper, the title of which is given in the catalogue at the end of this book, had come to the same conclusion, and that he had stated in addition the fact, that Koselanski had

proved the presence of reduced hæmoglobin in the blood of every dead body, if certain precautions for excluding the air had been taken. Hofmann concludes that the tissues of the body take the oxygen from the blood a few minutes after the lungs have ceased to convey air to that liquid. Hoppe-Seyler has also confirmed these observations, but Albert Schmidt has shown that there are exceptions to this condition in several kinds of death in warm-blooded animals. Thus, in death from breathing carbonic oxide (as will be referred to further on); in death from starvation or cold, in which the reducing power of the tissues is diminished; or in death from passage of air into the veins, the hæmoglobin is not reduced. But whether the blood retains the spectrum of oxidized hæmoglobin permanently, or only for a time, in death from these latter causes, has not yet been proved. Hofmann considers, and his conclusion is probably correct, that the difference is only of a temporary nature, for the blood has in itself the power of consuming its own oxygen, "in the absence of any contact with organic tissues." *

Spectrum of the blood in death from asphyxia.—Stroganoff endeavoured to decide the question, whether the blood of an asphyxiated animal contained oxy-hæmoglobin. He placed between two glasses the completely isolated jugular vein, or carotid artery of a rabbit, and compressed them sufficiently to allow of their being examined with the spectroscope. "It was invariably

* *E.g.* Blood corked up in a bottle soon becomes of a purplish colour, and gives the band of reduced hæmoglobin.

found on asphyxiating the animal that the blood, even at the last moment of the last cardiac contraction, always contained oxy-hæmoglobin." *But in death from asphyxia the blood, arterial as well as venous, immediately after death, gives the spectrum of reduced hæmoglobin;* the following experiment will prove the truth of this assertion. If a rabbit be asphyxiated by compression of the trachea, or by drowning, and if the blood be examined in the manner I have described, *within two minutes after death, or as soon as the blood can be examined, reduced hæmoglobin will be indicated by the spectroscope in the left auricle, left ventricle, and in the aorta.* This is an important fact, and the knowledge of it will be useful to medical jurists.

Spectrum of the blood in death from the prolonged inhalation of nitrous oxide.—Knowing that nitrous oxide has the power of reducing hæmoglobin in solution, in the same manner, it is said, as indifferent gases, such as nitrogen and hydrogen, I was anxious to determine whether the *arterial blood* of an animal poisoned by prolonged inhalation of this gas would give *immediately after death* the spectrum of *reduced hæmoglobin*. Accordingly a guinea-pig had nitrous oxide administered to it until it ceased to live, the blood was examined within two minutes from the time of death, and the *arterial blood* all over the body, and also the muscles, gave the spectrum of *reduced hæmoglobin*.

The fact of the muscles having yielded this spectrum is made more interesting, when the remark of Thudichum is remembered, viz. that in death during the collapse stage of cholera, the muscles were found by

him to yield the spectrum of reduced hæmoglobin ; thus explaining the symptoms, which are due to “suspended oxidation.” The spectroscope accordingly confirms the assertion made in ‘Wood’s Therapeutics,’ 1878, p. 268, that nitrous oxide produces its anæsthetic effects partly by stopping the supply of oxygen to the blood.* And it also suggests wherein lies the danger of its administration, viz. too prolonged inhalation ; for the *temporary* thus becomes converted into a *permanent* deprivation of oxygen.

Spectrum of the blood after death from the inhalation of carbonic oxide.—In examining the blood reduced by nitrous oxide we find that shaking with air brings back the spectrum of oxidized hæmoglobin, thus the gas did not enter into a combination with the hæmoglobin, it suspended its breathing power for the time ; but in carbonic oxide we have an agent which is quite different in its action, for it not only destroys the breathing power of the hæmoglobin, but it actually enters into a combination with it, displacing the oxygen volume for volume ; and so firm is the combination, that reducing agents fail to rob the carbonic-oxide-hæmoglobin of its carbonic oxide ; so that the spectrum of the combination is unchanged when sulphide of ammonium or other reducing agent is added to it. The spectrum, got by passing carbonic oxide (or even coal gas since it contains 7 per cent. CO) through blood, or through a solution of it, or by poisoning an animal with the gas, is characterised

* Of course this fact alone does not account for the anæsthesia produced.

by having two absorption bands resembling in their relative breadth, and their shading the bands of oxy-hæmoglobin, but differing from the latter in being nearer the violet end of the spectrum. Sp. 7, Chart I, represents the spectrum, and was mapped from a solution of blood obtained from the body of a mouse poisoned in an atmosphere of carbonic oxide. The colour of blood, or of a solution of blood, is made more scarlet after carbonic oxide is passed through it. When sulphide of ammonium, or when Stokes' fluid was added to the blood of the mouse, no reduction had taken place at the end of forty-eight hours, but instead, the band of sulphæmoglobin appeared in the red, which will be described further on. In death from the inhalation of the fumes of smouldering charcoal it is carbonic oxide which exerts its deadly influence, and the blood of people poisoned in this manner exhibits the bands shown in the map, and cannot be made to yield the spectrum of reduced hæmoglobin on the addition of reducing agents.

The blood of mice and other small mammals poisoned by being placed in an atmosphere of *coal gas*, gives the same spectrum; hence, if any doubt existed as to the cause of death in a given case where coal-gas poisoning was suspected, the spectroscope would at once decide the question. Not only has this instrument enabled us to detect carbonic oxide poisoning after death, but the study of carbonic-oxide-hæmoglobin spectra has suggested a treatment in those cases where life is not extinct. A common form of suicide on the Continent, more especially in Paris, is performed by

the intending victim shutting himself or herself up in a room from which all air is excluded, and then having lighted some charcoal, inhaling the fumes; hence it is as well to be aware of the treatment which is likely to be of use where life has not become wholly extinct. "Donders * states that carbon-monoxide may be expelled from blood saturated with it by oxygen, carbon dioxide, and hydrogen, even at 0°; oxygen does not convert the monoxide into dioxide, but simply drives it out. If this be the case, it should be possible to pump the carbon-monoxide out of blood saturated with it, although it may not be removed quite so easily as oxygen." The experiments of Zuntz † show that this is possible. "When blood saturated with carbon-monoxide was placed in a receiver connected with an exhausting pump, and warmed to 37°—42°, an active escape of gas took place, ceasing apparently at the end of half an hour. When, however, the pumping was continued at various intervals, fresh quantities of gas were given off, and a further quantity was obtained by heating the receiver to 60°. The blood so exhausted was found to give the spectrum of reduced hæmoglobin, which was replaced by the spectrum of oxy-hæmoglobin after standing in the air." These results did not coincide with those of other observers, because the latter were not able to extract the carbon monoxide from the blood by exhaustion, "inasmuch as the process was supposed to be complete when no more gas was evolved after the first pumping." So that in cases of poisoning by carbonic oxide artificial respiration should be

* 'Pflüger's Archiv,' iv, 28.

† Ibid., v, 584.

kept up vigorously for some time. It has also been proposed to treat such cases by the inhalation of oxygen, and it has actually been carried out, it appears with some success in Berlin. Professor Baeblich demonstrated the interesting fact that if oxygen be passed through carbonic oxide blood, it becomes reduced upon the subsequent addition of ammonium sulphide,* hence the reason of its adoption as an antidote.

Podolinski † has also shown that blood saturated with carbon-monoxide is completely deprived of that gas by agitation for half an hour with hydrogen, and more rapidly with oxygen. Nitrogen dioxide is also expelled by hydrogen, but less rapidly than carbon monoxide. Hence it appears that the compounds of hæmoglobin with carbon monoxide and nitrogen dioxide are similar in character to oxy-hæmoglobin; the order of stability being, oxy-hæmoglobin, carboxy-hæmoglobin, nitroxy-hæmoglobin. Each of the three gases, O, CO, NO, can be expelled by the one immediately following, and each also more easily by the one immediately preceding it, than by any other indifferent gas.

Carboxy-hæmoglobin can be obtained in the crystallized condition by passing a stream of the gas through an aqueous solution of hæmoglobin crystals, blood-corpuscles, or even defibrinated blood, cooling to 0°, mixing with $\frac{1}{4}$ vol. cold alcohol, and allowing the mixture to remain for some time at 0°. Bluish-red crystals form, less soluble and less easily

* 'Nature,' vol. xv, p. 362.

† 'Pflüger's Archiv,' vi, 553.

decomposable than those of oxy-hæmoglobin, but similar in form.

“According to Koschlakoff and Bogomoloff,* solutions of oxy-hæmoglobin and carboxy-hæmoglobin, through which *ammonia* is passed gradually, turn brownish-green and no longer exhibit any absorption bands. Arsine colours solutions of oxy-hæmoglobin first yellow-brown, then green-brown, the absorption bands gradually disappearing and being replaced by the band of reduced hæmoglobin, whereupon the solution becomes somewhat red; on the next day, however, the last-mentioned band disappears. On the other hand, carboxy-hæmoglobin is coloured dingy green by arsenic, and its absorption bands are destroyed.”†

Spectrum of blood treated with nitrogen dioxide.—

The oxygen of hæmoglobin is displaced by nitrogen dioxide, and the latter forms a new combination with the hæmoglobin. Arterial blood, however, takes up but a small quantity of the gas. “Thus the blood from the crural artery of the dog took up after addition of baryta water from 25·4 to 27·6 vols. per cent. of NO; defibrinated dog's blood 23 vols. per cent.; defibrinated ox blood 31·8 vols. per cent. (reduced to 0° and at 1 mètre pressure).”‡ Nitrogen dioxide (= nitric oxide) and hyponitric acid, give spectra very like that of oxy-hæmoglobin, but, as in the case of carbonic oxide, reducing agents fail to displace them from their combinations with hæmoglobin.

Spectrum of blood treated with sulphuretted hydro-

* ‘Zeitschr. Anal. Chem.,’ viii, 228.

† Watts, ‘Dict. Chem.,’ 2nd Supp., 1875.

‡ Watts, ‘Dict. Chem.,’ 1st Supp., 1872.

gen.—If sulphuretted hydrogen be passed through a solution of blood, the hæmoglobin first becomes reduced and then an additional band appears in the red. On shaking the solution with air, the broad band disappears and is replaced by those of oxy-hæmoglobin, but the band in red still remains; these appearances are shown in Chart I, Sp. 8 and 9.* And Chart III, Sp. 5, shows Dr Thudichum's map of blood treated with sulphuretted hydrogen; the latter map is different from that of mine in some respects, which will be observed when they are compared. To the body giving this spectrum which was first described by Nawrocki, the name sulphæmoglobin has been given by Lankester; it can be produced by adding excess of sulphide of ammonium to blood, especially if the latter be rather old, when the band in red appears quickly, but if it be fresh it only appears after some time, or with a large excess of the sulphide, or on being heated after the addition of the sulphide. Any alkaline sulphide according to Preyer produces sulphæmoglobin. If a strong solution of blood be corked up for three days with $\frac{1}{20}$ th its volume of the sulphide the spectrum will be obtained with great distinctness (Lankester). "I have carefully fixed its position, and find it to be quite distinct from that of any other hæmoglobin band," says Lankester in the 'Journal of Anatomy,' 1869, p. 119. He also observes that blood treated sufficiently

* I find that when ammonium sulphide is added to blood previously treated with sulphuretted hydrogen that we sometimes get the first band of reduced hæmatin within that of reduced hæmoglobin, and then those of reduced hæmatin (see methæmoglobin, cyanhæmatin, and nitrite blood), but the band in red still persists.

long with a sulphide gives eventually the bands of reduced hæmatin. I may here remark that this is an important observation, because it teaches the necessity of caution in using sulphide of ammonium as a reducing agent, for an excess of sulphide will not only develop the band of sulphæmoglobin, but after a little time it may,—if added to a product of hæmoglobin, such as methæmoglobin, intermediate between the former body and hæmatin,—bring out the bands of reduced hæmatin, and thus lead to an erroneous inference. “The previous addition to the blood of gallic acid and an alkaline carbonate has the same effect as putrefaction in making the sulphæmoglobin band occur at once when the sulphide of ammonium is added. This was pointed out by Professor Stokes, of Cambridge” (Purser). Preyer has noticed that sulphæmoglobin is not formed if the sulphuretted hydrogen is made to act on reduced hæmoglobin. Hence “water containing sulphuretted hydrogen can be drunk or injected into the veins without danger to life, while the danger of breathing the gas or of injecting its solution into the arteries is very great.”*

Solutions of hæmoglobin, free from oxygen, even in presence of ammonia, are not affected by sulphuretted hydrogen, but oxidized hæmoglobin, as stated before, is reduced, the first effect of this gas being the separation of the loosely combined oxygen from the hæmoglobin, this action being hastened by heat. In an ammoniacal solution of oxidized hæmoglobin abstraction of oxygen is the only action that

* Purser, after Preyer.

takes place, but in neutral solutions a band appears in the red. The colouring matter which gives this band differs from hæmatin and from methæmoglobin (of which a description is given further on) in this, that the solutions of the latter substances when treated with ammonia and ammonium sulphide exhibit certain bands in green, whereas the sulphæmoglobin,—got by passing sulphuretted hydrogen through a solution of oxyhæmoglobin,—remains unaltered when thus treated.* Hoppe-Seyler regards sulphæmoglobin as a sulphur compound of hæmatin and hæmoglobin. By the long-continued action of sulphuretted hydrogen this compound is decomposed, sulphur and an albuminous substance being separated, and another body is formed, which is olive-green in thin layers, and brown-red in thicker layers; this dries up into a brittle hygroscopic mass having a pitchy lustre. This substance is coagulated by heating its aqueous solution, as well as by acids and by alcohol. “It contains all the iron (0·44 p. c.) of the hæmoglobin, and about four times as much sulphur (1·57 p. c. instead of 0·415)” (Hoppe-Seyler). A solution of iron sulphide (as obtained with very dilute ferrous sulphate, tartaric acid, and ammonium sulphide) gives a band in red like that of the solution of hæmoglobin treated with oxygen and with sulphuretted hydrogen. But no formation of iron sulphide takes place in the above reaction on account of the presence of oxygen; besides, the product contains all the iron of the hæmoglobin.

Nawrocki† states that ammonium sulphide exerts on

* Not always, see note, p. 81.

† ‘Zeitsch. Anal. Chem.,’ vi, 285, and ‘Jahresb.,’ 1867, p. 802.

hæmoglobin at first a reducing, and afterwards a decomposing, action. A solution of hæmoglobin mixed with $\frac{1}{2}$ vol. of ordinary ammonium sulphide gives a dark band in the red at Fraunhofer's line C; the broad reduction band between D and E becomes narrower and more sharply defined, and afterwards a second broader band appears, covering E and extending beyond *b*. After the appearance of these bands, which disappear in about twenty-four hours, those of oxyhæmoglobin are no longer produced by agitation with air.

According to Preyer* potassium persulphide also causes the bands of oxyhæmoglobin to disappear, at first bringing out the band of reduced hæmoglobin, but afterwards, especially on heating gently, a sharply-defined black band, beginning at $\frac{7}{20}$ of the distance from D to E, and ending at $\frac{12}{20}$ of the same distance, and another band beginning at $\frac{17}{20}$ of the distance from D to E, and ending at $\frac{5}{8}$ of that from E to *b*, make their appearance. At boiling heat these bands disappear, the spectrum at the same time becoming shady, but they reappear if the solution be quickly cooled.

According to Hoppe-Seyler† potassium sulphide and ammonium sulphide act on hæmoglobin in the production of sulphæmoglobin only in the presence of free alkali, and act by producing and reducing hæmatin.‡

The spectrum of blood treated with hydrocyanic

* 'Jahresb.,' 1867.

† 'Medis. Chem. Unter.,' i, 299.

‡ I am indebted to Watts's 'Dictionary of Chemistry' for an account of some of these researches.

acid and other cyanides.—When cyanide of potassium, or when hydrocyanic acid, is added to a solution of blood and a gentle heat applied, the spectrum changes. Instead of the bands of oxyhæmoglobin we find a single broad band resembling the band of deoxidized hæmoglobin, but differing from it in two particulars, firstly, in being nearer to the violet end of the spectrum, and, secondly, in being most shaded on that side of it which is next the violet, the latter part of the spectrum being also obscured. See Sp. 21, Chart I. In stating this fact, which can be easily verified, I am not in accordance with some observers, who state that hydrocyanic acid has no effect upon blood, and that it is only with cyanide of potassium this spectrum is got; but as the map was drawn from an actual specimen, and as the result arrived at was constant after many experiments, the only essential being the application of a gentle heat, I prefer again reiterating the statement that hydrocyanic acid *does* affect the spectrum in this manner. With Scheele's prussic acid we get—if we add it in sufficient quantity—not this spectrum, but that of acid hæmatin; and I found that with the ordinary 2 per cent. acid of the 'British Pharmacopœia' a different result could be obtained according to the amount added.

By deoxidizing a solution of blood, in which the spectrum just described is well marked, we get two bands like the carbonic oxide bands; this spectrum is shown in Chart I, Sp. 22.

But this result is not always constant, for in using the 2 per cent. acid, and if after the broad band has

appeared we add a reducing agent we sometimes get a narrow dark band, in the position of the first one of reduced hæmatin, superimposed upon a broad band, in the position of that of reduced hæmoglobin. The reason of this is that too much hydrocyanic acid has been added, and the spectrum described above is entirely missed. In such cases it is probable that cyanhæmoglobin and cyanhæmatin are formed simultaneously, as compounds behaving like this are produced by other reagents as well as by hydrocyanic acid. And, further, if to blood which has been treated with hydrocyanic acid, and afterwards ammonium sulphide, and which presents this abnormal behaviour, we add before the addition of the ammonium sulphide, a little ammoniac hydrate we get the bands of reduced hæmatin. Hence the above solution of the problem is the correct one, for the ammonia converts all that part of the hæmoglobin which remains, into hæmatin, and so we only get the bands of reduced hæmatin when the sulphide of ammonium is added.

A concentrated solution of guinea pig's or dog's blood mixed with hydrocyanic acid, when $\frac{1}{4}$ th its volume of alcohol is added to it, and when it is cooled to 0° , deposits crystals which are exactly like those of oxyhæmoglobin, but they retain hydrocyanic acid even after repeated crystallisation and drying in a vacuum; the hydrocyanic acid can, however, be separated from them by distillation with water and a few drops of sulphuric acid (dilute). The compound of hydrocyanic acid with hæmoglobin crystallizes easily, but that of potassium cyanide does not. Neither com-

pound can be reconverted into oxyhæmoglobin; and neither can ozonise atmospheric oxygen.

When blood has been treated with cyanide of potassium and after the band described above has appeared, a stream of oxygen may be passed into the solution, without affecting the spectrum. According to Preyer, as stated before, ammonium sulphide develops two bands, the first extending from $\frac{5}{20}$ to $\frac{10}{20}$ of the distance from D to E, the other from $\frac{13}{20}$, between D and E as far as $\frac{3}{4}$ from E to *b*. So that this spectrum resembles that got by acting on blood with carbonic oxide. On acting upon the solution reduced by ammonium sulphide, with oxygen, the broad band reappears, and on adding ammonium sulphide repeatedly the first two bands are reproduced. Moreover, the solution can now be coagulated by heat, whereas the solution of blood acted on by hydrocyanic acid is not coagulated by heat.

It would appear from the behaviour of cyanide of potassium and of hydrogen cyanide with blood, and from the behaviour of these compounds with ammonium sulphide, that the latter contain oxygen which is removed by sulphide of ammonium, but which is more intimately combined with the hæmoglobin than in oxyhæmoglobin.

According to Nawrocki,* the broad band, got on adding hydrocyanic acid to blood, belongs to hæmatin, not to hæmoglobin; he shews that it is obtained at once and without the aid of heat, if the blood-solution is first mixed with caustic potash.

* 'Jahresb.,' 1867, p. 204.

Lankester found that cyanhæmatin was formed from cyanhæmoglobin if the latter solution had stood for two hours or more, "the pinkish-red colour being changed to an orange brown;" the cyanhæmoglobin solution was obtained by passing *cyanogen gas* into a solution of blood, and Lankester thinks that we may infer the existence of a compound between cyanogen and hæmoglobin, like CO-hæmoglobin, NO-hæmoglobin, &c. This view is opposed to that of Laschkewitsch,* as the latter states that cyanogen merely reduces hæmoglobin. "He is probably led to this conclusion by the observation of the cyanhæmatin of Hoppe-Seyler (having missed altogether the spectrum seen by me), which has a single broad band resembling, but quite distinct from, that of reduced hæmoglobin."† Professor Gamgee, in his report on physiology,‡ remarks: "Is it not likely that in this case a compound of the oxygenised blood colouring matter is formed with cyanogen, similar to the compounds with cyanide of potassium and with the nitrites, and that the spectrum described as that of reduced hæmoglobin is really the spectrum of the new substance?"

When potassium cyanide is added to an aqueous solution of blood which has been treated with carbonic oxide, the characteristic absorption bands do not disappear until the mixture has been heated to 40°, when the broad band of cyanhæmatin appears, and now on the addition of sulphide of ammonium the reduced

* Reichert's u. Reymond's 'Archiv,' 1868, p. 649.

† Lankester, in 'Journ. Anat. and Phy.,' November, 1869.

‡ 'Journ. of Anat. and Phy.,' May, 1869, p. 469.

cyanhæmatin bands appear; by agitation with air the broad band is reproduced, and afterwards the original spectrum of the carbonic oxide compound.

Hydrocyanic acid and ammonium cyanide act in the same manner with the aid of heat; but the filtered solution after agitation with air finally exhibits the bands of oxidized hæmoglobin.*

The blood of animals poisoned with hydrocyanic acid gives no characteristic spectrum, nothing beyond that of oxyhæmoglobin.

* Preyer, 'Zeitschr. Anal. Chem., vi, 289; 'Jahresb.,' 1867, p. 803.

CHAPTER V.

ABSORPTION SPECTRA OF BLOOD (*continued*).

Action of nitrites on blood.—The blood of animals, which have been made to inhale nitrite of amyl until the cessation of life, exhibits a chocolate colour, and when this blood is examined with the spectroscope, the change of colour is seen to be accompanied by a change of spectrum. There are three bands visible in the aqueous solution of such blood, the spectrum is shown in Chart I, Sp. 11.* This fact was first discovered by Professor Gamgee, of the Owens College, Manchester, and his paper bearing upon the subject will be found in the ‘Philosophical Transactions’ of the Royal Society of London for 1868, vol. 158, part II, p. [589]; he had previously published a paper on the action of the nitrites in the ‘Transactions of the Royal Society of Edinburgh’ (v. Appendix). After laying down a few propositions, Professor Gamgee shows that no one had hitherto investigated the action of nitrites on blood, his own attention having been first called to the matter by noticing the chocolate colour, which the blood of mice assumed when poisoned with the nitrite of amyl vapour. In his paper read before the Royal Society of Edinburgh

* A fourth band is also shown. The reason of this is explained in p. 97.

he had discussed the optical characters only, but in this he discusses the changes and influences which the nitrites exert on the relation of the blood to various gases. But here we shall only refer to the optical characters of the blood :

(1.) *As to colour.*—When defibrinated and well-arterialised blood is mixed with a solution of nitrite of potassium or sodium, its colour becomes almost immediately altered, changing to a chocolate brown. But the nitrites differ in the rapidity with which they act upon blood, for the blood of the dog is almost instantaneously affected, while the blood of the ox and sheep may take twenty minutes or even longer. The blood-cells of the dog are recommended for experimental purposes because they burst more readily. The nitrite should be dissolved in alcohol, and a few drops of the alcoholic solution added to the blood.

Ammonia in solution turns the chocolate-coloured blood of a red colour, which change was proved by experiment to have been independent of any alteration in the shape of the corpuscles.

(2.) *As to the spectrum.*—If after blood is diluted enough to show the bands of oxyhæmoglobin, we add a solution of the nitrites, when the solution begins to change colour to a brownish tint, the blood bands undergo remarkable changes. The two bands become fainter and fainter, and are only visible when a comparatively thick layer of the fluid is examined. At the same time if the layer be sufficiently thick, an additional though faint band appears in red. This band appears absolutely to coincide with that of acid hæmatin.

It is seen to best advantage where so thick a layer of solution is examined as to cut off all but the red rays. The complete change in spectrum is always coincident with the change in colour.

By the addition of ammonia to alkalinity the colour changes from chocolate-brown to blood-red again. Simultaneously the band in red disappears and the bands between D and E become again more distinct. In addition, that part of the spectrum which is between yellow and orange becomes shaded by a well-defined absorption band. According to Prof. Gamgee, the spectrum of the nitrite blood consists of three bands, which may be called, according to their amount of shading, δ , α , β .

δ is between C and D, near C.

α is shown covering D.

β occurs between D and E, near E.

The spectrum of the compound of nitrites with the hæmoglobin after the addition of ammonia showed a faint, and a darker band, touching each other, the dark one covering D, the light one on the red side of D, and a band between D and E. (In these investigations a single-prism spectroscope was used.) On adding sulphide of ammonium, or Stokes' fluid, to the nitrite blood treated with ammonia, an extraordinary change ensued. First of all, the spectrum of the nitrite blood treated with the ammonia appeared, which was then replaced by the spectrum of reduced hæmoglobin, and when the last was shaken with air the spectrum of oxidized hæmoglobin appeared.

Potassium nitrite also gave a band in red, and after the blood had been treated with this reagent, ammonia was added to the mixture. The band in red disappeared at once, and the two bands became more intense. The orange was again shaded, a faint band appearing to overshadow it, and on adding a reducing agent the oxyhæmoglobin bands appeared darker than ever, and after some time they gave way to the band of reduced hæmoglobin.

These experiments so far shewed—

(1) That nitrites exert a marked influence both on the colour and on the spectrum of blood, due obviously to a chemical change exerted on the hæmoglobin.

(2) This chemical change does not alter the composition of the colouring matter, as proved by the action of reducing agents.

(3) Nitrites neither expel nor remove the loose oxygen of the blood, because reducing agents develop the spectrum of oxyhæmoglobin before that of reduced hæmoglobin without the intervention of atmospheric oxygen.

The author then shews how nitrites modify the respiratory function of the blood by several interesting experiments, for which the reader may consult the original paper. His conclusions were as follows :

(1) When a solution of any nitrite acts upon blood, peculiar changes occur in the colour, and simultaneously in the absorption spectrum.

(2) These changes in the optical properties of blood are due to the formation of compounds presenting the same crystalline form, colour, and spectrum, whatever

the nitrite which has been employed in their preparation.

(3) These bodies appear to be compounds of the nitrite used, with oxidized hæmoglobin.

(4) The substances formed by this process of chemical addition, although isomorphous with hæmoglobin, differ from it in many of their most remarkable properties upon which its functions in the economy of the body depend. By this process of addition the blood-colouring matter appears to have lost its power of absorbing oxygen.

(5) The addition of nitrites to hæmoglobin appears to result in the locking up of the loosely-combined oxygen, so as to make it irremovable by carbonic oxide, or by a vacuum.

The author makes a few observations at the end of his paper which are worth quoting. "We have hitherto been acquainted with hæmoglobin itself, as well as with its O-, CO-, and N₂O₂-compounds. These compounds are all isomorphous, possess almost the same physical characters; in all the oxygen-free hæmoglobin has apparently linked itself to a molecule of O, CO, and N₂O₂ respectively, the stability of the compound being least in the case of the O-compound, and greatest in the case of the N₂O₂ compound.

"All these bodies, and pre-eminently the O-compound, appear to be examples of a class of bodies which stand, as it were, on the boundary line which separates chemical from physical combinations—to be, in fact, examples of the class of so-called molecular compounds. Like other molecular compounds, their com-

position varies greatly within certain limits, and is influenced by circumstances and conditions which have no action on chemical compounds proper.

“That a body possessing such a very complicated molecular structure as hæmoglobin should present numerous points of attachment, as it were, for the linking on of such active, condensed bodies as the nitrites, is more than probable, and it is not remarkable that, as in the case of other combinations of a molecular kind, such as the union of salts with their water of crystallisation, of bases with sugar, of albumen with metallic oxides, of iodine with the compound ammonias, the amount of the simple body added to the more complex should vary within wide limits.”

“The experiments of Hoppe-Seyler and of Preyer show that hydrocyanic acid possesses the property of linking itself to hæmoglobin, forming a body isomorphous with it, but which, physiologically, is an inert body, having lost the power which, normally, hæmoglobin seems to possess, of ozonising atmospheric oxygen.”

Professor E. Ray Lankester, on repeating these experiments, found, when sulphide of ammonium was added to the nitrite blood to which a little ammonia had been previously added, that the darker band of reduced hæmatin became visible in the midst of the broad band of reduced hæmoglobin; and on adding ammonia to the nitrite blood a clouding occurred in the position of the alkaline hæmatin band. “The band in the extreme red of the nitrite blood agrees

exactly with that of acid hæmatin, as Dr Gamgee observes. It therefore seems not improbable that, on addition of ammonia, a small quantity of hæmatin is really formed, which was partially developed on the first addition of the nitrite to the blood, as indicated by the band in red—but which does not separate—clinging, like the nitrite itself, in definite quantity, to the crystals of the blood thus treated” (Lankester). This appearance of Stokes’ reduced hæmatin band within that of reduced hæmoglobin would indicate probably the fact, that the spectrum observed was that of a mixture of hæmoglobin and hæmatin. I have observed exactly the same appearances in three instances : (1) in a solution of blood treated with 2 per cent. hydrocyanic acid when reduced ; (2) in the fluid vomited, in a case of hæmatemesis, when reduced ; and (3) in blood treated with sulphuretted hydrogen and then with ammonium sulphide. At the same time the spectrum of the nitrite blood is exceedingly like that of methæmoglobin, both as regards the position of the bands and in its behaviour with reducing agents.

I found, on repeating Gamgee’s experiments, but in a somewhat modified manner four absorption bands, the blood (that of the cat) had a few drops of amyl nitrite added to it, the characteristic change in colour very soon occurred, and on examining with the spectroscope three bands appeared, one near C, between C and D, one close to D, on the violet side of it, and another between D and E, slightly covering E, the violet end of the spectrum being shaded up to near *b*. On adding to this fluid, ammo-

nium sulphide I observed a band in the position of that of reduced hæmoglobin.

But if, after adding nitrite of amyl to blood, we shake the mixture with alcohol, a *four*-banded spectrum is obtained, three of the bands of which are evidently those of the aqueous solution, and the fourth band is placed between *b* and F.* The etherial solution is also characterised by having four bands, which differ slightly in their position and in their relative shading from those of the alcoholic solution. But that the bands of the etherial and of the alcoholic solution belong to the same chemical compound is proved by adding a reducing agent, as the latter soon develops a band in the position of that of reduced hæmoglobin. One curious fact which is observable about the nitrite blood is, that ammonia in solution develops a band in the same position as ammonium sulphide, but no further change beyond darkening took place in this band on adding a reducing agent to it.

It appears to me from what I have myself observed that the body formed by the action of nitrites on blood is very like, if not identical, with the body which will be next described, *i.e.*, methæmoglobin, because the spectrum of the latter not only closely resembles the nitrite spectrum, but also gives a band in the same place, that of reduced hæmoglobin, when reducing agents are added. It would seem that agents not sufficiently strong to split hæmoglobin up into hæmatin develop from it, or link themselves on to it

* It is best seen by illuminating the slit with direct sunlight.

to form compounds which are characterised by giving, upon the addition of reducing agents, the spectrum of reduced hæmoglobin. The spectrum got by treating fresh cat blood with nitrite of amyl and shaking with alcohol is shown in Chart I, Sp. 11.*

Methæmoglobin.—If a solution of hæmoglobin be left exposed to the air for some time it undergoes a change in colour, losing its brightness, and at the same time a change occurs in its spectrum, as a new band has appeared in the red; the same change occurs if solutions of hæmoglobin be evaporated at temperatures above 100° C.; and if the edges of a filtering paper, through which a solution of blood has been filtered, be examined with the microspectroscope the same band will sometimes be found. This band owes its presence to a brown colouring matter, which was called by Hoppe-Seyler methæmoglobin. It is said by some to resemble hæmatin in its optical characters, but this statement is incorrect, as solutions of methæmoglobin do not give the bands of reduced hæmatin, but that of reduced hæmoglobin when reducing agents are added to them. It differs from hæmatin also in the fact that it is soluble in water and in very dilute acids. A certain band in red, which is said by the authors of some books on the microspectroscope (and, indeed, by some physiological chemists) to be that of acid hæmatin, I find is really due to methæmoglobin, as the band of methæmoglobin can be produced

* The blood of an animal made to inhale the nitrite until it dies, gives the three bands described by Professor Gamgee, which differ slightly in position from those in the map.

by adding weak acids to solutions of blood. The same band can be made to appear by passing carbonic acid gas through dilute solutions of hæmoglobin, or by adding a very small quantity of glacial acetic acid to solutions of blood. Permanganate of potassium causes a like transformation in hæmoglobin. If a crystal of the permanganate be dissolved in water and added to a very dilute solution of blood before the slit of the spectroscope, and kept at a temperature of 25° C., the hæmoglobin bands gradually disappear, and a new spectrum appears instead, which has not only the band in red, but also two* others, which occur nearly in the position of the hæmoglobin bands.

The fact that methæmoglobin is often present in pathological fluids makes it especially interesting to the student of medicine. I have found it in the urine of acute desquamative nephritis, in that of post-scarlatinal nephritis, and in the blackish-brown fluid vomited in some cases of hæmatemesis. The spectrum of a solution obtained by the action of potassium permanganate on blood is shown in Chart I, Sp. 10, and the spectrum of the same body was noticed in the fluid vomited in a case of hæmatemesis. In the last case, in addition to methæmoglobin, hæmatin was also probably present, as sulphide of ammonium developed the first band of Stokes' acid hæmatin within that of reduced hæmoglobin. There is a danger of confusing methæmoglobin with sulphæmoglobin, but the

* A fourth band is sometimes seen in some pathological fluids; it occurs to the violet side of the third band mentioned above. The fourth band, which is described under the nitrite spectrum will represent its position. See Chart I, Sp. 11.

latter is distinguished from the former by not losing the band in red on adding ammonium sulphide to its solutions.

The chemical nature of methæmoglobin is not accurately determined, but some have considered it a hyperoxide of hæmoglobin; Hoppe-Seyler* shows that this view is incorrect, since in some cases where methæmoglobin is found, the fact of any oxidation having taken place is out of the question. It is more correct to assume that methæmoglobin is a mixture of hæmatin with a soluble albumen, and from what has been already said about it, this appears to be the most rational view.

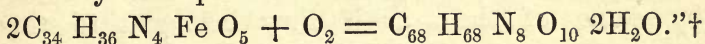
Action of ammonia gas, arseniuretted and anti-moniuretted hydrogen on hæmoglobin.—According to Koschlakoff and Bogomoloff when ammonia gas is passed into solutions of oxy-hæmoglobin or CO-hæmoglobin, the solution assumes a yellow colour, the bands disappear, and are not replaced by that of reduced hæmoglobin or those of hæmatin.

Arseniuretted hydrogen, according to the same authorities, and also according to Thudichum, causes the appearance of the reduced hæmoglobin spectrum when passed into solutions of O-hæmoglobin. When passed into a solution of CO-hæmoglobin, this gas exerts a similar action to NH_3 and PH_3 , *i.e.* the entire disappearance of the absorption-bands. When it is passed into alkaline solutions of hæmatin the bands of reduced hæmatin appear.

* 'Zeitschrift für Physiol. Chemie,' ii, p. 148, and 'Centralblatt f. Med. Wiss.,' January 25th, 1879.

Antimoniuretted hydrogen exerts a similar action on O-hæmoglobin and on CO-hæmoglobin.*

Hæmatin and hæmochromogen.—Before discussing the spectra of hæmatin it will be necessary to refer to a fact which was discovered by Hoppe-Seyler, and published in 1871. It was considered up to that time that by the action of acids and alkalies the hæmoglobin of the blood was split up into a colouring matter *hæmatin*, and an albuminous body *globin*, but it appears from Hoppe-Seyler's discovery that hæmatin is not a *direct* product of the splitting up of hæmoglobin, but results from such a decomposition *accompanied by oxidation*. "This oxidation takes place so rapidly, that it is only by special precautions that the non-oxidised products can be obtained. When, however, a solution of hæmoglobin is reduced by hydrogen and decomposed by alcohol containing sulphuric acid or caustic potash, in an apparatus from which oxygen is completely excluded, a colouring-matter is produced, which is acid, has a purple-red colour in alkaline solutions, and is characterised by certain definite absorption bands." This is *hæmochromogen*, which yields hæmatin by oxidation. "It has not yet been isolated or regenerated by reduction of hæmatin, but its spectrum agrees generally with that of reduced hæmatin. Hoppe-Seyler supposes it to have the composition $C_{34}H_{36}N_4FeO_5$, and represents the formation of hæmatin from it by the equation :



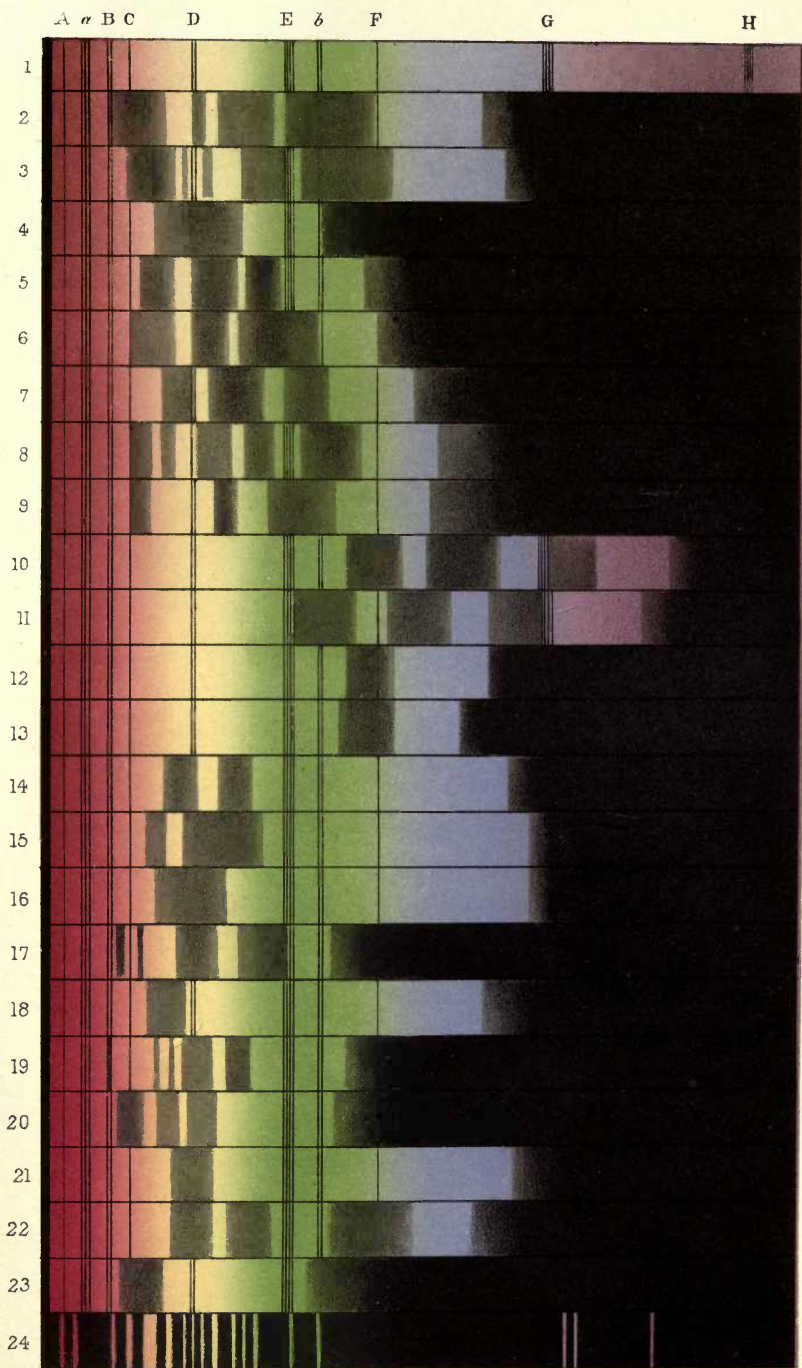
* Gamgee, 'Journ. of Anat. and Physiol.,' 1869.

† Watts' 'Dict.,' 2nd supp., 1875. This equation does not show what becomes of Fe.

Hæmatin.—Although hæmatin has been already referred to, the consideration of its spectrum and of the methods of procuring the different kinds of hæmatin has been purposely deferred till now. The most complete account that I know of the various kinds of hæmatin is given by Thudichum in the ‘Tenth Report of the Medical Officer of the Privy Council,’ which is referred to in the Appendix. I have drawn in Chart III, all the most important spectra given by that author in the plates accompanying his paper, and I have myself repeated his experiments, and thus can vouch for their accuracy. After an account of his methods and those of others, a brief summary of easy methods, by which all the important spectra of hæmatin and cruentin can be procured, will be given, but it was not until after I had studied Dr Thudichum’s methods, that I was able to procure the various decomposition-products of hæmoglobin, by simple and rapid processes. Judging, therefore, from my own experience, I believe his methods ought to be more generally known, and I must also take this opportunity of observing that he has not been sufficiently thanked by the members of his own profession for the great and valuable additions to our knowledge of physiological and pathological chemistry which he has made. To German chemists and to others who have made absorption spectra their study, his methods are well known, and his results have but too frequently been made to appear by some of them as if they belonged to themselves.

“*Blood treated with Alcohol and Ammonia.*”—Sp. 6

CHART III.



Chas. A. MacMunn fecit.

Hanhart lith.



Chart III, represents the spectrum of a solution of blood in alcohol and ammonia which was thus prepared. "Blood was mixed with twice its volume of alcohol, and the coagulum filtered from the liquid, the latter showed no spectrum bands. The coagulum dissolved in ammonia showed three bands which had a general resemblance to the sulphuretted hydrogen blood spectrum (Sp. 5, Chart III), but showed different measurements; when to this Stokes' fluid was added the spectrum of reduced hematine appeared." I have also given a drawing of the spectrum, which I obtained by pouring alcohol and ammonia on to some defibrinated cat's blood, a method somewhat different from Dr Thudichum's; this spectrum is shown in Chart I, Sp. 14, and gave the reduced hæmatin bands when treated with ammonium sulphide.

"*Spectral Phenomena of Hematine.*"—"When hematocrystalline is treated with acids, or alkalies and alcohol, it is split up into albuminous substances, and a coloured matter, which retains all the iron, but none of the sulphur of the original compound. This is hematine. It appears from my researches that there are at least three different kinds of hematine recognisable by the spectroscope, besides a number of combinations which may be formed by one or perhaps all of them."

"*Five-banded Hematine.*"—"Blood-corpuscles isolated from serum by sulphate of soda, are treated at the ordinary temperature with alcohol to which a little sulphuric acid has been added. This solution was found by me to possess five absorption bands, three of

them being those of acid hematine, first described by Stokes, two others being situated in red and orange, and narrow, one very fine, like a narrow bundle of sun-lines" (Chart III, Sp. 3).

"*Four-banded Hematine by a modified Process.*"—"When the corpuscles are boiled in water after treatment with sodium sulphate, so that all sulphate is removed, and are then treated with acidified alcohol, a solution is obtained which gives a spectrum similar to the" last in three of its bands, but having one fine line in the orange instead of two. "This is identical with the spectrum of hematine first described by Stokes. It was also obtained by dissolving pure crystallised hematine in alcohol and a little sulphuric acid by the aid of a gentle heat" (Chart III, Sp. 2).

"*Blood treated with Acid.*"—"The simple addition of acid to blood changes its spectrum. When an organic acid, such as acetic or tartaric, is taken, one band in red appears, and great obscuration of the rest of the spectrum ensues. The one band in red belongs to acid hematine, and the darkness in green is due to two other bands. These, first correctly described by Stokes, it is not easy to define without the aid of sunlight or Drummond's light. They have, therefore, not been noticed by later authors. Upon the purest specimens of acid hematine they can, however, be observed and measured with tolerable accuracy. The acid solution of hematine was hitherto believed to contain a particular body, which was termed hemine. It is, however, quite easy to show that it is hematine, for its spectrum can, by alternate acidification and

alkalification, be made to yield the bands either of acid or alkaline hematine."

"*Alkaline Hematine.*"—"In order to fully study the phenomena of hematine, I produced a quantity of it in a neutral state. A large quantity of amorphous hemato-crystalline was made from ten gallons of blood and a hundredweight of potassium carbonate. The isolated material was dried at 40° C., and extracted with cold absolute alcohol. The splendid ruby red solution was treated with a solution of tartaric acid in absolute alcohol as long as a precipitate fell down. The filtered solution was then slowly evaporated at 40° C. until it deposited all colouring matter as a fine powder of black, somewhat violet colour. This was filtered off, the powder washed with alcohol, lastly with water. It was then redissolved in absolute alcohol and potassium carbonate. Tartaric acid was again added and crystallisation completed as before. Ultimately a black violet powder remained, consisting, under high powers of the microscope, of little rhombic scales, mostly crossed, and imitating well the shape of the hemine crystals. I believe this to be pure crystallised hematine. It certainly contains no hydrochloric acid, and negatived the assumption hitherto made by some animal chemists, that all crystallised hematine was identical with hemine, and like hemine, was a hydrochlorate of hematine.

"The neutral hematine is insoluble in water, alcohol, and ether, but dissolves in caustic alkaline water and in alkaline or acid alcohol. The spectrum of the acid solution is that just described." The pure hematine,

prepared as above, gave, when dissolved in alkaline alcoholic solution, one broad band covering D (see Chart III, Sp. 4). Treated with a little sulphuric acid, the spectrum became that of acid "hematine."

"*Reduced Hematine.*"—"When an alkaline watery solution of hematine is mixed with a deoxydising agent, such as the alkaline tartrate solution of ammonio-sulphate of iron suboxyde," the spectrum gave the bands of Stokes' reduced hæmatin (see Chart I, Sp. 15).

"*Cruentine, a new derivative of Hemato-crystalline and of Hematine.*"—"When human or animal hemato-crystalline is boiled with sulphuric acid it becomes chemolysed, the albumen dissolves and yields its particular products, a portion of the hematine also dissolves and colours the fluid ruby red, while a brownish red, grumous matter remains suspended in the fluid in an insoluble state. This is a mixture of neutral cruentine with its sulphate. By washing with water, this matter loses sulphuric acid, and becomes ultimately free from it. Treated with sulphuric acid it dissolves completely, and is now sulphate of cruentine."

"*Cruentine Sulphate.*"—A concentrated solution of this body gives one black band in red to orange, the blue end of the spectrum being shaded. On dilution this band splits up into two, "and a third very feeble band in green becomes visible just to disappear." Chart III, Sp. 7, shows this spectrum.

"*Neutral fluorescent Cruentine.*"—"The insoluble residue from the sulphuric acid treatment is washed to neutrality and dried, a portion of it is soluble in ether

and chloroform. The ether solution has four bands which are a little less shaded, but nearly identical with the bands of the chloroform solution." (My own drawing of the spectrum of the latter is shown in Chart I, Sp. 18.) This solution of cruentine fluoresced "with a splendid blood-red colour in the sun cone. This is the first body which is known to fluoresce with homogeneous light, that is to say, the same kind of light or colours which it transmits."

"*Alkaline four-banded Cruentine.*"—The solution of cruentine in alcohol is made alkaline by ammonia, and gives the spectrum which I have figured in Chart I, Sp. 17,* from a solution which I prepared by the method I shall describe.

"*Neutral five-banded Cruentine in Alcohol.*"—"When the preparation from which chloroform extracts the neutral fluorescent cruentine is treated with alcohol it dissolves easily and almost entirely. The concentrated solution allows a little red to pass. On further dilution three bands appear, ultimately five, one in red feeble, one in yellow, also feeble, both narrow, and three dense and dark bands in green." Sp. 8, Chart III, represents this spectrum.

"*Reduced Cruentine.*"—This spectrum was got by adding Stokes' solution to an ammoniacal solution of cruentine in alcohol. It is shown in Sp. 9, Chart III. On acidifying this solution with sulphuric acid, a precipitate fell, and after filtration, the filtrate gave the bands of cruentine sulphate. "Cruentine, therefore, exhibits this peculiar property, that it can be deoxydised (in

* I found five bands.

alkaline) and reoxydised (in acid) solutions. During this process, however, much colouring matter is lost by changes not yet scrutinised. In its alkaline solution it is deoxydised and reoxydised as easily as hemato-crystalline. It is a most remarkable fact in science that a decomposition product of hemato-crystalline of the second order retains what I will term the breathing power of the blood-corpuscle."

"*Cruentine and Hydrochloric Acid.*"—"The chloroform solution treated with HCl and water becomes turbid. Warmed, it clears up and appears more rose coloured. On cooling it becomes, however, again turbid. Its spectrum shows three bands," which are figured in Chart I, Sp. 19.

These are the principal blood spectra described by Thudichum. Other observers call by different names the products which he describes.

General account of hæmatin and its reactions.—The foregoing account of the methods of Thudichum, has shown, how the substances which have been called by English observers alkaline and acid hæmatin, and that called by the author just quoted cruentine, can be prepared, but what he has said is not sufficient to enable any one who has not read the subject before to understand it thoroughly, so that I shall give a short account of what authorities say upon this matter. A short repetition of what has been said before may be pardoned, as it is unavoidable.*

* Thudichum holds opinions exclusively his own on hæmatin and cruentin, and he does not agree with Preyer or Hoppe-Seyler in their views as to the composition of hæmatin.

Hæmatin was at one time supposed to be the colouring matter of the blood, but Hoppe-Seyler showed that it does not exist preformed in that fluid, but is produced, together with globin, an albuminous body, by the action of acids and alkalies on the hæmoglobin of the blood.* It may, according to this authority, be obtained pure by dissolving its hydrochloride (hæmin) in ammonia, evaporating to dryness, heating the residue to 130° , dissolving out the ammonium chloride by water, and again heating to 130° . It may also be obtained by mixing defibrinated blood with a strong solution of potassium carbonate until the liquid adhering to the separated coagulum becomes colourless, drying the coagulum at a temperature not above 50° C., and digesting it for some days with absolute alcohol in a close vessel at a moderate temperature (below 50°). The red liquid, when filtered, is an alcoholic solution of hæmatin.

In acid liquids the spectrum is a four-banded one, if an alcoholic or etherial solution be used, and differs in no essential respect from that which is got by merely acting upon blood with acetic acid and shaking up with ether, which I shall describe.

The brown solution of hæmatin in potash or potassium cyanide exhibits least absorption of light near C; on diluting this solution there remains a band between D and E, but nearer to D, which, however, disappears while the solution still exhibits a strong colour.

According to Nawrocki an alkaline solution of

* Hæmochromogen, an intermediate product being first formed. See p. 101.

hæmatin (hæmin crystals) dissolved in ammonia gives a spectrum with a broad band between C and D, but after treatment with a ferrous salt, or with stannous chloride, it shows two other bands, which do not disappear on agitation with air, and are likewise visible for some time in the red ethereal solutions obtained by mixing the ammoniacal liquid with ether and glacial acetic acid, but in this they merge into the three bands of the normal hæmatin solution. If, on the other hand, the alkaline solution of hæmatin be mixed with ammonium sulphide, the liquid exhibits the same bands as hæmoglobin when similarly treated, and no longer yields up anything to ether on addition of acetic acid (?).

“Hæmatin or hæmin heated for some time with ammonia, or a fixed alkali, is converted into a body, the solution of which in acidulated alcohol, or in an alkali, has a dingy olive-green colour, dark red in thick layers, and after treatment with reducing agents does not exhibit the spectrum of reduced hæmatin, neither can hæmin crystals be obtained from it.”*

Iron-free hæmatin.—“By dissolving hæmin in strong sulphuric acid, and adding water to the solution, a substance is precipitated resembling hæmatin, but not containing iron; it is soluble in alkalies. The solution of this non-ferruginous hæmatin in strong sulphuric acid absorbs blue and violet light strongly; on diluting it with sulphuric acid a very dark, well-defined band appears about midway between D and E, and a narrow band between C and D (near D), the spectrum being

* Hoppe-Seyler, quoted in Watts' 'Dictionary.'

very darkly shaded between D and the dark absorption-band. The solution of non-ferruginous hæmatin in dilute ammonia exhibits the smallest absorption for red light. On diluting with water a band appears between C and D, and on further dilution three others. Reducing agents alter this fluid in the same manner as ordinary hæmatin.”* It is quite evident that the body whose spectrum is thus described is the same as the substance called by Thudichum “cruentine,” which the latter author has shown to contain 1·51 per cent. of iron. On the other hand, Foster states that “by the action of sulphuric acid hæmatin may be robbed of all its iron.”

Preyer maintains† that the body which goes into solution when blood is treated with acetic acid and shaken with ether, which Stokes called acid hæmatin, is really free from iron, and he calls it hæmatoin. But, if acid hæmatin is free from iron, so also ought alkaline hæmatin to be, as I have found that there are some acids which when added to blood give a four-banded spectrum, that can be made to give the same spectrum as that yielded by alkaline hæmatin on the addition of reducing agents.‡ The long names *hæmatoporphyrin* and *hæmatolin* have been proposed for bodies, the former of which appears to be practically identical with cruentin, from the description of its spectrum by Hoppe-Seyler.§ It is got by filtering a solution of hæmatin in oil of vitriol through

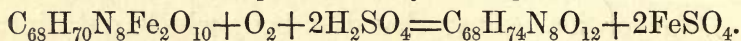
* Hoppe-Seyler, 1865.

† ‘Die Blutkrystalle,’ p. 181.

‡ This, however, is merely a spectroscopic reason.

§ ‘Med. Chem. Unter.,’ 523, 1871.

asbestos, when a fine purple-red solution is obtained, which gives a small, dark absorption-band just before the line D, and another sharply-defined band between D and E. When this solution is mixed with water the greater part is precipitated, the precipitation being increased by the addition of alkalis to neutralisation. The alkaline aqueous solutions give a faint band between C and D, another faint band between D and E, nearer D, a dark band in the same interval near E, and a dark band between *b* and F. This substance, *hæmatoporphyrin*, is free from iron, and gives by analysis 68.42 p. c. C., 9.58 N., 6.07 H., and 15.93 O. Its formation is represented by the equation :



When, on the other hand, hæmatin is acted on by sulphuric acid in closed vessels, hæmatolin, $\text{C}_{68}\text{H}_{78}\text{N}_8\text{O}_7$, is formed, which is but very slightly soluble in sulphuric acid, and very slightly soluble in caustic potash.

Hæmatin hydrochloride.—In describing the process by which Hoppe-Seyler recommends hæmatin to be procured, hæmin was incidentally mentioned. This substance has been known under the name of Teichmann's crystals, and has assigned to it the formula $\text{C}_{96}\text{H}_{142}\text{Fe}_3\text{O}_{18}$. It is obtained in regular crystals by treating hæmoglobin or methæmoglobin with common salt and glacial acetic acid. The crystals are rhombic or six-sided plates, dark blue by reflected, dirty brown by transmitted, light; insoluble in water, alcohol, and ether; soluble in acids and alkalis, but decomposed by all acids except acetic and hydrochloric.

It may be prepared as follows:—Defibrinated blood dried at the ordinary temperature, or blood clot cut up and dried, is powdered in a mortar with one fifth part pure carbonate of potassium, and the dried mass digested with alcohol of 94 per cent. at 40° — 45° until the resulting dark, garnet-coloured solution no longer becomes darker in colour. The solution is filtered; the residue again treated with alcohol; the united extracts mixed with rather more than an equal volume of water, and then with enough acetic acid to produce a slightly acid reaction. The brown flocculent precipitate which is produced is collected on a filter and dried slowly, the heat being finally raised to 100° ; it is triturated with one fifth part sodium chloride and from twenty to thirty parts glacial acetic acid, and the mixture digested for some time at 60° until a crystalline mass separates. The whole is heated to 100° and left to cool; the crystals are then washed on a filter with warm glacial acetic acid, pressed, dried, and again boiled with water (J. Gwosden).

Hoppe-Seyler has modified this process; he causes a coagulum to separate from the blood by pouring it into alcohol or boiling water; the clot, separated by filtration, and still moist, is warmed with alcohol to which a few drops of strong sulphuric acid have been added; the filtered brown solution is mixed with a warm, saturated solution of sodium acetate, then immediately neutralised with sodium carbonate, and, in order to separate the hæmin, if this has not already separated, it is mixed with water, or freed from alcohol

by distillation. The precipitate, after being washed on a filter and dried in the air, is then ready for treatment with common salt and acetic acid, as in the former method. Although this substance is of no interest *directly* to the spectroscopist, it is *indirectly*, as it has been used to prepare pure hæmatin, accordingly I considered that its mode of preparation ought to be mentioned.

The exact amount of sodium carbonate required to convert hæmoglobin into hæmatin.—When a small quantity of sodium carbonate is added to a solution of hæmoglobin, no *coagulation* takes place, even after the fluid has been heated to 100° C. At 54° C. the substance is obviously decomposed, as this solution becomes of a dark, brown-red colour, and examined with the spectroscope it exhibits the spectrum of hæmatin in alkaline solution, instead of that of oxyhæmoglobin. The fluid is alkaline and remains clear after boiling. At the temperature at which the decomposition takes place, it is probable that hæmoglobin splits up into hæmatin and albumen, and Preyer calculated the amount of sodium carbonate which had to be added in order to prevent the coagulation by heat; the mean of two observations showed that one gramme of hæmoglobin in distilled water required 0·0238 gramme of the carbonate. Accordingly, he concludes that one molecule reacts on three molecules of hæmoglobin in order to produce the non-coagulating compound (Gamgee).

The action of various acids on hæmoglobin in producing hæmatin.—Preyer has studied the action of the following acids on hæmoglobin :

Phosphoric.	Acetic.	Lactic.
Phosphorous.	Formic.	Citric.
Sulphurous.	Butyric.	Tartaric.
Oxalic.	Propionic.	Malic.
Monochloracetic.	Metaphosphoric.	Succinic.
Phosphomolybdic.	Benzoic.	Carbolic.
Gallic.	Hydrochloric.	Uric.
Nitric.	Hippuric.	Sulphuric.
Pyrogallic.	Carbonic.	Chromic.

All act somewhat similarly, but some of them precipitate the hæmoglobin, and upon the occurrence of a precipitate and its character Preyer bases a classification of their action. The action of alkalies and alkaline solutions is more uniformly similar than that of the acids.

Action of carbonic oxide on hæmatin.—Dr L. Popoff has studied the action of carbonic oxide on solutions of hæmatin, and has arrived at the following conclusions :

(1) Carbonic oxide causes no change in acid or alkaline solutions of hæmatin when passed through them.

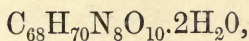
(2) If, however, a reducing agent had been added at the same time that the carbonic oxide was being passed through the solution, a new compound was formed, which, in the case of an ammoniacal solution of hæmatin, was deposited in the form of a flocculent red precipitate.

(3) The spectrum of this consists of two bands similar to, and identical with, those of reduced hæmatin.

Action of tin and hydrochloric acid on hæmatin.—

Tin and hydrochloric acid act on hæmatin in the presence of alcohol differently, according as the hæmatin is fully dissolved or not. When a concentrated solution, or one containing excess of hæmatin, is heated with tin, copper, or zinc, on the water bath, a purple-red colour is produced, and after a time a resinous, dark violet precipitate forms. The solution gives two dark bands between D and E. Hæmatin, or hæmin crystals, dissolved in alcohol containing sulphuric acid, when decomposed by hydrochloric acid and tin and heated, give a purple solution, which has a characteristic spectrum. One band between D and E, another before D, but close to it, and a broad band between *b* and F, covering the latter line.

Hæmatin treated with phosphorous chloride containing free phosphorus.—When hæmin crystals are heated with this substance to a temperature of 104° in closed tubes for from six to eight hours, a purple-brown fluid is obtained, which gives three absorption bands, one between C and D, close to C, another between D and E, near E, and a third between *b* and F. No gas escapes on opening the tube, but a crust forms on the sides of the tube, which is easily separated. Part of this is soluble in water, and the aqueous solution gives the same spectrum as hæmatoporphyrin. From that part which is insoluble in water a substance can be got resembling hæmatin, but containing phosphorus. The agreement of the spectrum of this compound with that of hæmatoporphyrin seems to show that the latter consists of—



or that it is a hydrate of the same molecule as that contained in the phosphorus compound (Hoppe-Seyler).

Action of ammonia, arsine, and stibine on hæmatin.

—Ammonia (gas) colours alkaline solutions of hæmatin orange, and the absorption bands become paler; a broad but diffuse shadow appears in the green part of the spectrum, and after a little time an amorphous precipitate forms. This precipitate dissolves in acetic acid, and the solution gives the spectrum of acid hæmatin. When arsine is passed into alkaline solutions of hæmatin, the colour of the liquid gradually becomes red, and the bands of reduced hæmatin appear. Shaking with air restores the colour of the alkaline hæmatin, but after a few seconds the solution begins quickly to redden, and this alternation may be repeated about ten times. If the arsine be passed for a longer time, the alkaline solution turns brown, and may deposit next day crystals of arsenic of a steel-grey colour. After this time no more bands are seen; nevertheless, the presence of hæmatin may be demonstrated by means of reducing agents. The bands of reduced hæmatin may be recognised at a much greater degree of dilution than those of the alkaline or of the acid solution, a fact which seems to show that, in spite of the action of the arsine, part of the hæmatin has remained undecomposed. The action of stibine is the same as that of arsine (Koschlakoff).

CHAPTER VI.

EASY METHODS OF PREPARING THE MOST IMPORTANT OF THE SPECTRA WHICH HAVE BEEN HITHERTO DESCRIBED; AND APPLICATION OF THESE METHODS.

ANY one reading the last chapter might be led to suppose that the study of the blood-spectra is an exceedingly difficult one, but such is not the case; and I shall now describe how all the spectra of importance can be prepared by very rapid and very simple processes; and, in addition, other spectra will be mentioned which have not been hitherto described.

Oxidized and deoxidized hæmoglobin have already been mentioned, so that their consideration need not be repeated.

Alkaline hæmatin.—Make a saturated solution of carbonate of potash in alcohol, and pour a few drops of blood into the solution, the colour of the blood immediately changes, and when examined the spectrum shown in Chart I, Sp. 13 is seen, a broad, lightly-shaded band covering D. The action of caustic alkalies on defibrinated fresh cat blood was found to give different spectra from those which are often described, thus:

(1) *Caustic potash and caustic soda* gave, in alcoholic solution, when added to blood, each the same spectrum, but different, in some respects, from that got from ammoniac hydrate and from carbonate of potash.

It consisted of three bands, two of which were like the blood bands, but that the product giving this spectrum was hæmatin was proved on adding a reducing agent, as the spectrum of reduced hæmatin now appeared.*

(2) An *ammoniacal* solution of alcohol was now added to a few drops of blood (the same blood as before) when a faint band in red, and a dark band nearer violet appeared. On further dilution the latter was seen to be composed of two, and on adding a reducing agent the bands of reduced hæmatin appeared (see Sp. 14, Chart I). The feeble band in red has not been generally noticed, and I believe the reason of this is, that observers generally work at old blood in which the hæmoglobin has been reduced.† I often found a great discrepancy arise when the same experiment was performed respectively on old pig blood, or old ox blood, and fresh blood removed from the body immediately after death.

Reduced hæmatin.—This can be got by adding Stokes' fluid, or ammonium sulphide, to the solution of blood treated by alcohol and carbonate of potash, or by the alcoholic solution of caustic soda, of caustic potash, or of ammonia; but if an alkali *alone* be added to blood previously, the reducing agent will *not* develop this spectrum.

The reduced hæmatin spectrum can be also got from the hæmatin formed by the action of *some* acids on hæmoglobin, thus, from that got by acting on blood

* This spectrum is so easily procured that it is unnecessary to give a map of it.

† The band in red is only seen with the microspectroscope.

with salicylic acid and dissolving the hæmatin formed in alcohol, we can, by adding ammonium sulphide, get the spectrum of reduced hæmatin; in the same way the alcoholic solution of blood treated by lactic acid can be made to yield the spectrum of reduced hæmatin. Sp. 15, Chart I, is that of reduced hæmatin.

Acid hæmatin.—Add a few drops of acetic acid to blood and shake with ether; the brown-red ethereal solution gives four well-marked bands. This spectrum is shown in Chart I, Sp. 12. The feeble band near D is with difficulty seen at that degree of dilution, which shows the bands in green to best advantage. In examining with the microspectroscope, it is necessary to narrow the slit sufficiently and focus carefully, and in working with the chemical spectroscope it is a good plan to move the telescope from side to side, when a faint shadow, in the position of this feeble band, will be seen to move across the field of view. By *looking obliquely*, too, feeble bands are sometimes seen with the latter instrument which would otherwise be missed.

Action of bromine on blood.—This spectrum was first described by me in the ‘Dublin Journal,’ June, 1877.

(1) Ox blood treated with bromine and shaken with alcohol gives four absorption bands, which are practically identical with those of acid hæmatin.

(2) Ammonium sulphide added to this gives, not only the bands of reduced hæmatin, but a third feeble band in orange, close upon D (see Chart I, Sp. 24).

(3) If ammonia be added to the first solution it develops a band at D, like that of alkaline hæmatin.

(4) Ammonium sulphide added to this develops the same bands as in (2).

(5) A solution of blood treated with an aqueous solution of bromine gives a band just like alkaline hæmatin, or that of (3). (Chart I, Sp. 23.)

(6) When ammonium sulphide is added to this it gives exactly the same spectrum as that got in (2) and (4). Thus, bromine teaches an important fact, viz. that a different kind of hæmatin is produced according to the *amount* of chemical action on hæmoglobin. It would appear from its action that alkaline hæmatin is a body which is produced from hæmoglobin by a less complete "splitting up" than in the case of acid hæmatin. This form of hæmatin is, however, different from that of ordinary hæmatin, as it gives three bands, instead of two, on the addition of reducing agents. The compound is insoluble in chloroform, sparingly, or not at all, in ether, slightly in absolute alcohol, but more freely in rectified spirit.

Action of iodine.—Added to blood an aqueous solution (with iodide of potassium) produced a precipitate, and the supernatant fluid gave no spectrum, although there was evidently a little hæmatin present in solution, since the addition of ammonium sulphide gave the bands of reduced hæmatin. The precipitate, when dissolved in alcohol, gave no spectrum. Chlorine seems to act much in the same manner, by forming an insoluble form of hæmatin.

Sulphate of cruentin.—Boil defibrinated blood with strong sulphuric acid, add water and filter, wash the mass on the filter until the washings are neutral to

test paper, and dry. This can be used for the preparation of all those kinds of cruentin which Dr Thudichum describes. Dissolve some in sulphuric acid, it gives a fine ruby-red colour, giving two absorption bands; this is the spectrum of sulphate of cruentin (Sp. 16, Chart I).

Alkaline cruentin.—Dissolve some of the neutral dried precipitate of the last experiment in alcohol and ammonia when a red fluid will be obtained, giving five (or four) bands (Sp. 17, Chart I).

Neutral cruentin.—Dissolve some of the neutral dried precipitate in chloroform, this gives four bands, but differing in their positions from those of alkaline cruentin (Sp. 18, Chart I).

Hydrochloric product of neutral cruentin.—Add hydrochloric acid to the last fluid it becomes turbid, heat, the turbidity disappears; it is now of a purplish colour, and gives three bands (Sp. 19, Chart I).

Reduced cruentin.—Add ammonium sulphide to the alkaline solution of cruentin; this solution gives three bands, which are shown in Chart I, Sp. 20. It is noticeable that the darkest band of this spectrum is nearly in the same place as the first reduced hæmatin band.

Methæmoglobin.—Add a solution of permanganate of potassium to a solution of blood; notice the position of the band in red. This is the only band of importance; it is shown in Sp. 10, Chart I, in which the other bands are also seen. Dilute and add ammonium sulphide; notice the band of reduced hæmoglobin.

Sulphæmoglobin.—Pass sulphuretted hydrogen for some time through a solution of blood; in this case also a band in red is developed, but there is also a broad dark band in the position of that of reduced hæmoglobin. (See Chart I, Sp. 8.)* Add ammonium sulphide, and notice that the band in red persists, thus presenting a contrast to methæmoglobin, in which the band in red disappeared.

Cyanhæmatin.—Add a solution of cyanide of potassium to a solution of blood and *heat gently for some time*; the blood bands disappear and a band is developed which has been already described, p. 85. (See Chart I, Sp. 21.) Add a reducing agent, and notice the appearance of two other bands nearly in the position of the blood bands, but nearer the violet (Chart I, Sp. 22).†

CO—Hæmoglobin.—Pass coal gas through a solution of blood for some time, the solution becomes red and looks clearer than before; examine with the spectroscope, two bands are seen nearer the violet than the blood bands. Add ammonium sulphide, no effect is produced (Chart I, Sp. 7).

Pathological application of the study of the spectra of hæmoglobin and of hæmatin.—Having learned the appearances of the various decomposition products of hæmoglobin, the reader will be now in a position to

* On shaking with air this band is replaced by two. (Sp. 9.)

† Having repeated these experiments several times I can promise that, if any one will take the trouble to repeat them for himself, he will have no difficulty in obtaining the spectra which are shown in Chart I; and, indeed, without actually performing them for himself, he will not be in a position to draw any inferences from the spectra of pathological fluids.

examine pathological fluids, and by studying the action of reducing agents, to draw correct inferences by means of the spectroscope. There are certain spectra which ought to be most familiar to the medical spectroscopist, viz. that of hæmoglobin, oxidized and reduced, that of methæmoglobin, that of acid, and of alkaline and reduced hæmatin.

Blood which has not been kept in contact with an acid secretion for too long a time, as in hæmorrhage from the bladder or urethra, gives the spectrum of oxidized hæmoglobin in the majority of cases.*

In hæmorrhage from the kidney, when the hæmorrhage is gradual, and a result of inflammation, the spectrum of methæmoglobin will generally be seen.

In hæmoglobinuria, intermittent cruenturesis, or paroxysmal hæmaturia, or, as it has been incorrectly called, hæmatinuria, the spectrum is generally that of methæmoglobin.

When the blood has been acted on by the acids of the gastric juice, as in various forms of hæmatemesis, the spectrum of methæmoglobin, with a mixture of acid hæmatin, will be found; for on adding sulphide of ammonium to such fluids I have sometimes found the band of reduced hæmatin within that of reduced hæmoglobin.

If the blood has been shut up within a cyst and kept at the temperature of the body for a considerable time, it may, as in the case of ovarian and par-ovarian cysts, have been converted into a form of

* Provided the urine be examined soon after removal from the bladder.

hæmatin, this can be proved by the addition of sulphide of ammonium.

Blood in the urine.—Blood may be present either in a soluble or insoluble state. It may readily be detected when present in the soluble condition by holding the vessel containing it between the eye and the source of light, and examining with an ordinary pocket spectroscope; if it contains blood two bands are visible as shown in Chart I, Sp. 4; if in the form of methæmoglobin, a band will be seen in red in addition to the other two. (If no blood be present a shadowy band may be noticed between green and blue, that of urobilin, which will be referred to again). To prove that the band in red is not due to acid hæmatin, some of the urine is placed in a test-tube and a reducing agent (sulphide of ammonium) added, when if the band be due to methæmoglobin, the spectrum of reduced hæmoglobin will appear, if to acid hæmatin, that of reduced hæmatin. (Or the reducing agent may be added in a small cell beneath the microspectroscope.)

But the blood may be present in an insoluble state; in which case the urine (both before and after filtration) may show no absorption bands. In this case, filter the urine, digest the filtering paper with the deposit upon it in alcohol and ammonia, and examine the fluid with the spectroscope; there may be the faintest possible shadow in the orange if there is but little blood present, but on adding ammonium sulphide, the first dark band, and occasionally the second band, of reduced hæmatin at once show themselves; shake up with air, the bands disappear, again to reappear on standing. The same

direction will apply in the case of other fluids supposed to contain blood. A small blood-clot, the nature of which is doubtful, may be treated in the same way, or it may be digested in alcohol acidulated with sulphuric acid, and the spectrum of the solution examined, which will be found to be that of acid hæmatin.

Hæmatin in parovarian and ovarian cysts.—Thudichum states that the contents of certain kinds of ovarian cysts give the spectrum of lutein, but he does not say what kind of cyst gives the spectrum, and he does not notice the very important fact that a kind of hæmatin is sometimes present in parovarian and ovarian cysts, which if it should be peculiar to these cysts, will be a great help in diagnosis. I discovered this fact, thanks to the kindness of Mr Lawson Tait, of Birmingham, who has taken the greatest trouble in sending me specimens. Three specimens of parovarian fluid, and one of ovarian out of five, gave the same spectrum; the fifth was almost colourless and gave no spectrum whatever.

The first specimen of parovarian fluid was olive brown in very thin layers, in deeper, blackish brown, and in very deep almost black, with a greenish shade. Before the slit of the chemical spectroscope, the fluid itself gave a band in red, and two in green, one of which latter was sufficiently distinct to allow of its being mapped. One band was placed between C and D, nearer C, another between D and E. (See Sp. 20, Chart II.) *On adding ammonium sulphide to this fluid the bands of reduced hæmatin appeared at once.* (The sulphide of ammonium was quite

pure* and its action on hæmoglobin previously tested.) Acetic acid caused the band in red to become less distinct, and ammonia intensified it. There might have been a feeble band close to F, but this was not quite certain.†

Microscopic examination, with a $\frac{1}{12}$ th immersion, showed crystals of cholesterin in great abundance, leucocytes, granular round bodies, very large round granular bodies which kept rolling about, no crystals of any kind beyond the cholesterin; very few blood-corpuscles. The reaction was alkaline (due to fixed alkali). Specific gravity determined by bottle 1024·6. The fluid gave no precipitate with acetic acid, but a copious one with nitric acid, and with nitrate of silver soluble in ammonia (chlorides). It was coagulable by heat, the precipitate when burned smelling of burnt feathers.

A quantitative analysis gave the following result :

Water	.	.	90·346
Solid organic matter	.	.	8·736
Inorganic salts	.	.	·918
			<hr/>
			100·000

The inorganic salts consisted of chlorides, sulphates, phosphates, and carbonates of sodium, potassium, and calcium. *Iron* was also present, probably from the hæmatin.

* This is a matter of the greatest importance, as disappointment will be experienced with changed sulphide.

† In some specimens a fourth band—close upon D—appeared. Compare Sp. 12, Chart I.

The next specimen of parovarian fluid was of a brown colour with a tinge of yellow round the edges in thin layers, while it was dark brown in deeper layers. It gave exactly the same spectrum as the first specimen, both in the original condition, and on the addition of ammonium sulphide.

Its reaction was faintly alkaline and its specific gravity was 1012·6. Under the microscope there was an absence of cholesterin, but the same large round granular bodies as before. No blood-corpuscles, but refracting granules were visible. It evidently contained paralbumin, since in using the test of Koeberle this was proved to be present; *i.e.* a precipitate with nitric acid partially soluble in acetic acid. It gave no precipitate with a solution of tungstate of sodium acidulated with acetic acid.

The third specimen was ovarian fluid, it was turbid, and of a dirty-brown colour; its reaction was feebly alkaline, and specific gravity 1013. Its spectrum was identical as regards the position of the absorption bands with those of the other two specimens, and on the addition of reducing agents the spectrum of reduced hæmatin again appeared. A remarkable change in the spectrum, and even in the colour of this specimen was found to have taken place after the lapse of twenty-four hours, for it then showed a band between C and E, slightly covering D, and another at F, the latter, however, being feebly marked; it is shown in Sp. 22, Chart II. On adding to the solution—giving this last spectrum—ammonium sulphide, the bands of reduced hæmatin became visible.

The microscopic and chemical characters of this specimen were almost the same as those of the last one.*

Here, then, we have a fluid which, although alkaline in reaction, yet gave the spectrum of acid hæmatin, and which in the last case gave a band very like that of alkaline hæmatin after standing some time; it is not impossible that while in the body the reaction was acid, but that after exposure to the air decomposition had set in and an alkaline reaction was developed, which went on increasing until the fluid was sufficiently alkaline to convert its contained acid hæmatin into the alkaline variety, as shewn by its changed spectrum. The examination of this fluid teaches the importance of a knowledge of the action of reagents upon hæmoglobin, for no one could have imagined that acid hæmatin, which was supposed to be only capable of being produced *out of the body* by the action of acids on the blood, could have been produced actually *in the body* by the action of reagents furnished by the human body itself.

I believe that the spectroscope will enable the contents of ovarian and parovarian cysts to be diagnosed, from other fluids resembling them, in many cases, but even if it should not of itself be able to do so, we have other corroborative tests to fall back on, such as that of Koeberlé—a precipitate with nitric acid which is soluble in acetic acid. I would refer the reader to the 'London Medical Record' for 1876, p. 269, for an account of his researches.

* Hence the spectroscope will not enable parovarian to be distinguished from ovarian cysts.

The presence or absence of the band of lutein will not help the diagnosis, as serous fluids yield the spectrum of this body (see last Chapter).*

The application of the study of the absorption bands of hæmoglobin and hæmatin to Medical Jurisprudence.
The detection of blood-stains.—As Mr Sorby is the great authority on this subject, I here give an abstract of a paper of his which appeared in the ‘Monthly Microscopical Journal’ (1871, vol. vi, pp. 9—17), entitled “On some Improvements in the Spectrum Method of Detecting Blood.”

“There does not appear to be any probability of our being able to decide, by this means, whether blood is, or is not, human.”†

The spectrum microscope used in these inquiries should have a compound prism, with enough, but not too great, dispersive power, or else the bands become, as it were, diluted, and made less distinct.

Cells, &c.—The cells should be made from barometer tubing, and be about one eighth of an inch in internal diameter, and half an inch long, one end being fastened to a piece of plate glass with purified gutta percha, like an ordinary cell for mounting objects in liquids. It is, however, of great advantage to insert between the plate and the cell a diaphragm of platinum foil, having a circular hole about two thirds of the internal

* There is also another test for paralbumin, which, with the microscopic character of the deposit from these fluids, will be found described in Thomas’s ‘Diseases of Women.’

† Krauss (‘Jahresb.,’ 1861) states that hæmin crystals from human blood are different from those got from the blood of oxen, sheep, pigs, and poultry.

diameter of the tube, fixed so that its centre corresponds with that of the cell. This prevents any light passing upwards that has not penetrated through the whole length of the solution, which is very important when using direct concentrated sunlight to penetrate through turbid or very opaque liquids. A small spatula made of stout platinum wire, flattened at the end, is very convenient for adding small quantities of the reagent; and they should be stirred up in the cells with a platinum wire flattened and turned up at the end, like a small hoe.

Reagents required.—A diluted solution of ammonia, citric acid; double tartrate of potash and soda, the last being used to prevent the precipitation of oxide of iron, and the double sulphate of the protoxide of iron and ammonia employed to deoxidise. In some special cases dilute hydrochloric acid, purified boric acid, and sulphite of soda are required.

Character of stains and action of reagents.—The character of a stain varies with its age, and with the nature of the substance on which it occurs. If quite recent, and if the substance has no immediate influence on blood, the stain contains little or no colouring matter but hæmoglobin. This is easily dissolved in water, and when sufficiently diluted it gives the spectrum of oxy-hæmoglobin, which, on the addition of ammonia, and of a small quantity of the double tartrate, and then a small piece of ferrous salt, about $\frac{1}{40}$ th of an inch in diameter, and stirring carefully, avoiding access of air as much as possible, changes to the spectrum of reduced hæmoglobin. When stirred

so as to expose the solution as much as possible to the air, the two bands again appear. On gradually adding citric acid in small quantities until the colour begins to change, these bands slowly fade away, and if there had been much blood present a band appears in the red. When previously deoxidised, this solution may be turbid, but not so as to interfere with the result. The addition of excess of ammonia makes all clear again, but does not restore the original bands, or only to a slight degree, for the hæmoglobin has been changed into hæmatin. This reaction alone distinguishes blood from most coloured substances, which latter, after being changed by acids, are restored by alkalies to the original state. On adding the ferrous salt to the ammoniacal solution we get the spectrum of reduced hæmatin (Chart I, Sp. 15), though, if the quantity of blood be small, only the first of the two bands may be seen. If too much citric acid or double tartrate had been added this solution might be turbid, but if all had been properly managed it would be quite clear. As the deoxidisation takes place slowly, especially in cold weather, it is well to slightly stir up the ferrous salt at the bottom, completely fill up the cell, cover it with a piece of thin glass, remove the excess of liquid with blotting paper, and mix the solution by turning the tube upside down over and over again. On reoxidising the solution by stirring, the bands of deoxidised hæmatin disappear, and the two bands of hæmoglobin will probably be recognised, owing to citric acid not changing the original merely into hæmatin, but also giving rise to some methæmoglobin.

The whole of these facts may be seen with a single cell containing about $\frac{1}{100}$ th of a grain of blood. Very faint bands are best seen by lamplight. Exposed to the air in a damp place, a blood-stain may be completely decomposed by the growth of mould, but when not thus destroyed, it is partly changed into hæmatin. If it had been kept dry the hæmoglobin has become changed into a variable mixture of methæmoglobin, hæmatin, and a brown substance not yet much studied. This change takes place more rapidly in the acid atmosphere of towns and houses, especially when gas is burned, than in the open country; but it does occur even in the purest air, and in glass tubes hermetically sealed. The presence of a weak acid in perspiration may also cause a stain on a worn garment to be completely changed in a very short time, and the presence of a stronger acid on dirty clothes may at once alter the hæmoglobin into hæmatin.

On digesting a stain in which all the hæmoglobin has changed into methæmoglobin from being kept a long time, the methæmoglobin dissolves. When the solution is sufficiently strong, this shows a band in red, and two fainter bands in green. The addition of ammonia removes that in red, but makes those in green much darker, and develops a special, very narrow band in orange. Deoxidised, this gives the spectrum of reduced hæmoglobin. Since methæmoglobin is formed at once from hæmoglobin by the action of a great number of different oxidising agents, and since it can be reconverted into oxidised hæmoglobin by slight deoxidation, Mr Sorby is inclined to

look upon it as a peculiar oxidised modification. On adding a little of the double tartrate and of the ferrous salt to even a dilute solution from an old stain, the methæmoglobin is deoxidised, and the well-marked spectrum of fresh blood can be seen. If left too long, the spectrum of deoxidised hæmoglobin is developed, but on well stirring, that of the oxidised reappears, and the various other spectra may be obtained as described above. That part of the stain insoluble in water, which is chiefly hæmatin, may be dissolved in dilute citric acid or ammonia, and when deoxidised the spectrum seen to even greater advantage than when fresh blood is employed, because there is no general shading in the green due to there having been methæmoglobin mixed with the hæmatin. We may thus obtain an excellent spectrum from a blood-stain nearly fifty years old. In very old stains all the methæmoglobin has disappeared, and sometimes even a considerable part of the hæmatin has been altered into another brown colouring matter, which does not give any well-marked spectrum.

When a blood-stain has been made sufficiently hot to coagulate the albumen, neither water, citric acid, nor cold ammonia will dissolve it, but by heating in dilute ammonia the hæmatin is easily dissolved, and may be detected either before or after concentrating the solution by evaporation. The spectrum of deoxidised hæmatin can in no way be better seen than by deoxidising a solution of fresh blood that has been boiled with dilute ammonia, which gives rise to a very pure hæmatin.

Examination of the stain.—In applying these principles to the detection of suspected stains, it is desirable, in the first place, to examine a portion of the unstained fabric, to ascertain whether any colour is dissolved from the fabric by dilute citric acid or dilute ammonia, and if so, to determine whether this would in any way interfere with the recognition of blood by the processes described above. In the case of scarlet cloth, and of some other red fabrics, much colour is dissolved out by ammonia, but not by citric acid, which ought, therefore, to be used; whereas, in other cases, ammonia is the best solvent.

Unless the stain be faint, a portion should be soaked in a few drops of water in a watch-glass, the liquid squeezed out, allowed to stand a short time in the glass, so as to deposit any small portion of the fabric, and poured into one of the experiment cells. If the stain had been recently made, and had not been changed by any special action, a solution of hæmoglobin would be obtained, and the various spectra could be seen one after the other, as already described. If, however, the stain were a few days, or a few weeks old, we should obtain a mixture of hæmoglobin and methæmoglobin, or the latter alone. The various spectra could then be developed, and compared, side by side, with those from fresh blood, to be sure that there is complete correspondence in the position and relative intensity of the bands. The residue insoluble in water should then be dissolved in dilute citric acid or ammonia, according to the nature of the fabric, and the spectrum of deoxidised hæmatin developed. If insoluble

in cold citric acid or ammonia, hot ammonia should be tried, since the stain might have been so heated as to coagulate the albumen. If it be desirable to keep the specimen of deoxidised hæmatin for subsequent reference, the cell may be covered with a piece of thin glass, and after removing the excess of liquid, the edge of the cover painted round with gold size. When properly managed, such an object will show a perfectly good spectrum, even after many weeks.

The most important absorption spectra in detecting blood stains.—If, therefore, we have a sufficient amount of a moderately old stain, we may easily see, in succession, the seven very different spectra of the following solutions :—(1) Neutral methæmoglobin ; (2) alkaline methæmoglobin ; (3) deoxidised methæmoglobin ; (4) oxidised hæmoglobin ; (5) acid hæmatin ; (6) alkaline hæmatin ; (7) deoxidised hæmatin. If the amount was very small, only (4) and (7) would show distinct bands, and the rest would be characterised rather by their comparative absence ; and it must always be borne in mind that (1) and (2) may be modified by the presence of unaltered hæmoglobin, (3) by that of dissolved hæmatin, and (5), (6), and (7) by that of undecomposed hæmoglobin or methæmoglobin.

Mr Sorby considers that these spectra afford as satisfactory a test for blood as could be desired.

Examination of faint blood-stains.—The foregoing directions apply to simple cases, when there is enough material at command, and when the fabric on which the stain is found does not contain anything which makes the blood insoluble, or interferes with the various

tests. If the stain be very faint, from the presence of but little blood, or from partial removal by washing, it might be necessary to examine the whole at once. In this case the stained portion should be digested in a few drops of dilute citric acid or ammonia and the presence of hæmatin determined, as already described. If faint and spread over a considerable surface, it might be well to digest in citric acid or ammonia diluted with much more water than would fill the experiment cell, and the solution afterwards concentrated by gentle evaporation. By this means blood could be detected, even when considerable effort had been made to remove it, and only a faint brown tinge left, just visible on white linen. There would generally be no difficulty in the case of a stain on cloth which had been sponged, for enough blood solution would be left in the fabric.

The effect of mordants in the detection of blood-stains.—

The presence of mordants in cloth or prints may require a modification of these proceedings, especially if the stains had been wetted, and to a great extent removed, so that we have only the dried-up solution of blood, thoroughly incorporated with the mordant. In the case of a piece of brown cloth, portions of which with a wetted stain were sent by Mr Sorby to a number of the highest authorities who pronounced it impossible to recognise blood on it, he found that after the lapse of six years, the presence of blood could be detected, by digesting a portion of the cloth in dilute ammonia and squeezing it over and over again with a pair of forceps, and finally with the finger and thumb,

so as to obtain as much of the solution as possible. This solution was very turbid, but when deoxidised in the usual manner, and illuminated by concentrated light direct from the sun itself, the band of deoxidised hæmatin was quite distinct. When the cell was kept for some time, so that the insoluble part settled to the side, no band was visible, and therefore the hæmatin was evidently combined with the mordant. He therefore recommends the solution in such cases to be examined with a sufficiently strong light, and the sediment not to be allowed time to settle. If the sun could not be made use of, the lime or electric light would, no doubt, be the best substitute.

Effect of vegetable soil on blood-colouring matter.—When fresh blood solution is agitated in a test-tube with vegetable soil, and left until quite clear, the colouring matter is completely carried down with the earth. Dilute ammonia, however, dissolves out hæmatin, and therefore, in testing portions of soil, they should be digested in considerably more of that solvent than will fill an experiment cell, and after the solution has become quite clear it should be concentrated by evaporation. The spectrum of deoxidised hæmatin may then be seen by following the ordinary method. The same process should be adopted in examining stains on clothes impregnated with earth or earthy dust, and marks on iron contaminated with much rust, if water will not dissolve out the unaltered blood or methæmoglobin.

Detection of blood-stains on leather.—The importance of being able to detect blood-stains on leather was pro-

minently brought before Mr Sorby by a case in which the trial of a suspected person depended on the nature of certain dark marks on his gaiters. The presence of tannic acid so completely mordants the blood, that neither water nor citric acid will dissolve it, and ammonia gives rise to a most inconveniently dark solution. If the stain is on the surface, and has never been wetted, a thin shaving should be cut off, so as to have as much blood, and as little leather as possible, and the blood should be dissolved off without exposing the solution to the action of the leather itself. This may be accomplished by taking one of the experiment cells, nearly filled with water, bending the shaving, and inserting it into the upper part of the water, so as to touch the water, being careful to arrange it so that the stain may be on the convex side of the leather, and in contact with the water. When a drop of blood falls on leather, many red globules are filtered out from the serum and left on the surface, and when thus treated, they dissolve, and the coloured solution sinks at once to the bottom of the cell, without coming into contact with the leather. The various spectra may then be observed in the usual manner. This method would be of little or no use if the stains had been wetted, and for a long time Mr Sorby concluded that after such treatment it would be impossible to recognise blood. However, after many experiments, and after having again and again almost given up the inquiry in despair, he found that the difficulty could be overcome in a very simple manner. The best solvent for the insoluble compound of the colouring matter of the

blood with tannic acid, is hydrochloric acid diluted with about fifty times its bulk of water. If stronger or weaker the result is not so good. When a portion of unstained common brown leather is digested in this dilute acid, the solution is scarcely tinged yellow. On adding excess of ammonia, the colour becomes pale purple, or neutral tint, made deeper when the double tartrate and the ferrous salt are added, but remaining nearly clear. This gives a spectrum very dull all over, but without any trace of definite bands in any part. The depth of colour varies much with different specimens of leather. A portion of similar material soaked with wetted blood gives a yellow solution, made brown-purple and turbid by the double tartrate and ammonia, and remains so when deoxidised. The band of deoxidised hæmatin can, however, be distinctly seen with a light sufficiently strong to penetrate the turbid and dark solution.

Before examining the suspected stain, it would be well to make out how much of the unstained leather could be used without giving too dark a solution, and to use no more of the stain. If the deoxidised solution be too turbid, the cell may be kept for a while horizontal, until the deposit has subsided sufficiently to allow the principal absorption-band to be seen; but it is not so distinct, when all has subsided, as though the greater part of the hæmatin still existed as a compound insoluble in dilute ammonia.

The presence of tannic acid in wood and other substances might make it necessary to employ a similar process, if the relative amount of blood be so small

that none could be dissolved out by water, or dilute citric acid.

Precautions necessary when other coloured matters besides blood are present.—Cases might occur when it would be necessary to decide whether blood were present, along with some other coloured substance soluble in water. The method to be employed would depend much on the nature of this impurity. If it were a colouring matter belonging to those pigments in which the absorption is removed by sulphite of soda, in an alkaline solution, there would be no difficulty in seeing all the spectra. Thus, for example, it is easy to add so much magenta to the solution of a little blood that its absorption bands are entirely hidden; but a small quantity of sulphite of soda so completely removes the colour of the magenta, that the various spectra of the blood may be seen almost as well as if it had been pure. If the colouring matters are those of fruits, the presence of free acid would be almost certain to have changed the hæmoglobin into hæmatin. The best plan would then be to add excess of ammonia, and, if the solution was made too dark, to dilute it with so much water that the strongest light at our command would show the green part of the spectrum sufficiently bright to prove that no absorption band occurred there. On deoxidising in the usual manner the solution may be made somewhat darker by the presence of tannic acid, but the dark band of deoxidised hæmatin could be recognised without material difficulty.

Cochineal is a colouring matter that requires special



attention. The addition of ammonia to its solution in water gives rise to two bands in the green, which though differing materially from those of blood, are yet so nearly in the same situation, that they completely disguise the presence of a small amount of blood. However, on adding a small excess of boric acid, the bands of the cochineal are made more faint, and very considerably raised towards the blue end, so as to leave the red end of the green clear, whilst those of oxy-hæmoglobin are not changed, and by that means the red end, if not both, can be seen perfectly well. By proceeding in the usual manner there is no great difficulty in recognising the darker band of deoxidised hæmatin.

Other difficulties which may occur.—We need never despair of detecting blood so long as any hæmatin remains undecomposed. Fortunately it resists decomposition so well, that this would rarely happen in ordinary circumstances; but yet there are cases in which it does occur, as, for example, when acted on by strong ozone, or other powerful oxidising reagents.

It is quite possible that stained garments might have been washed, and some of the water employed might be obtained. If no soap had been used, this water could be examined in a long tube of thick glass, ten inches or more in length, and a quarter of an inch in internal diameter, permanently closed at one end with a circular piece of plate glass, and, when filled, covered over at the other with another glass. A pocket spectroscope is the best instrument for using in this examination, such as that made by Mr Browning. If

only two or three days old, the bands of oxidised hæmoglobin might be seen; but if the solution had been kept longer, and these bands could not be detected, it should be concentrated by evaporation at a gentle heat, and tested for hæmatin. If during evaporation any deposit be formed, insoluble in cold dilute ammonia, it should be dissolved by the aid of heat. When soap has been used to wash off a stain, the alkali of the soap has converted the hæmoglobin into hæmatin, and the soap has made the solution inconveniently turbid and opaque. It is best in such a case to agitate the suspected soap and water with ether, remove it with a pipette, after the two liquids have completely separated, and repeat the process over and over again with fresh ether, until the aqueous solution at the bottom has become quite clear and free from soap. It should then be concentrated by evaporation, and examined for hæmatin, as usual. Of course, in such cases it would be desirable to test the solution as soon as possible, lest decomposition should occur; but by these means a very small quantity of blood, that would show no colour, might be recognised within a week or two, but probably not after.*

Richardson's method of detecting blood-stains.—In the 'Monthly Microscopical Journal' for 1876, vol. xv, pp. 30—32, Dr Joseph G. Richardson, of Pennsylvania, describes his method of detecting blood

* In the foregoing pages I have given almost the exact words of Mr Sorby, changing the sentences slightly here and there for the sake of brevity; and I have also divided the paper under separate headings, so as to facilitate reference.

in medico-legal cases. He says : " The value to medical jurisprudence of spectrum analysis, as employed for the detection of dried blood, is so fully established by the researches of H. G. Sorby, Dr W. B. Herepath, Professor A. S. Taylor, W. Preyer, and others, that it seems unnecessary for me to do more than state that the demonstration of the two dark bands in the green, caused by scarlet cruorine (hæmoglobin), such as that contained in a recent blood stain, enables experts to discriminate positively blood from other red colouring matters soluble in water, whether mineral, vegetable, or animal, except an extract of the red feathers from the *Turacus Albocristatus*, a bird found in the East Indies, and quite unknown on our continent of America. Valuable as this test is thus seen to be, there are, unfortunately, several circumstances which limit its general application, as, for example, the changes in the constitution of hæmoglobin which occur from prolonged, and frequently from comparatively brief, exposure to the air, the modification of the absorption bands caused by the presence of other substances, and last, but not least in many instances, the difficulty of procuring sufficient material for experiment. The insuperable nature of this latter obstacle will be at once appreciated when I mention that whilst the smallest amount which Sorby, Herepath, and Taylor furnish directions for is a spot 'one tenth of an inch in diameter, or a quantity of the red colouring matter amounting to no more than one thousandth part of a grain,' the important stain upon an axe handle, supposed to have been used in a murder,

I am now investigating, probably weighed less than one three thousandth of a grain when entire and uninjured." Dr Richardson then goes on to describe the method he adopted which enabled him to reveal the presence of blood in a quantity of matter "one hundredth the amount directed by Mr Sorby." "Procure," he says, "a glass slide with a circular excavation in the middle, called by dealers 'a concave centre,' and moisten it around the edges of the cavity with a small drop of diluted glycerine. Thoroughly clean a thin glass cover, about one-eighth of an inch larger than the excavation, lay it on white paper, and upon it place the tiniest visible fragment of a freshly dried blood clot (this fragment will weigh from one twenty-five-thousandth to one fifty-thousandth of a grain). Then, with a cataract needle, deposit on the centre of the cover, near your blood spot, a drop of glycerine about the size of this period (.), and with a dry needle gently push the blood to the brink of your microscopic pond, so that it may be just moistened by the fluid. Finally, invert your slide upon the thin glass cover in such a manner that the glycerined edges of the cavity in the former may adhere to the margins of the latter, and, turning the slide face upwards, transfer it to the stage of the microscope.

"By this method it is obvious we obtain an extremely minute quantity of strong solution of hæmoglobin, whose point of greatest density (generally in the centre of the clot) is readily found under a $\frac{1}{4}$ -inch objective, and tested by the adjustment of the spectroscopic eyepiece. After a little practice it will be

found quite possible to modify the bands by the addition of sulphuret of sodium solution, as advised by Preyer.

“In order to compare the delicacy of my plan with that of Mr Sorby, a spot of blood one-tenth of an inch square may be made on a piece of white muslin, the threads of which average one hundred to an inch. When the stain is dry, ravel out one of the coloured threads and cut off and test a fragment as long as the diameter of the filament, which will, of course, be a particle of stained fabric measuring one one-hundredth of the minimum-sized piece directed by Mr Sorby. When the drop of blood is old, a larger amount of material becomes requisite, and you may be obliged to moisten it with aqua ammoniæ, or with solution of tartrate of ammonium and protosulphate of iron; but in the criminal case referred to, *five months* after the murder, I am able, from a scrap of stained muslin, one-fiftieth of an inch square, to obtain well-marked absorption bands, easily discriminated from those produced by a solution of alkanet root with alum, and those caused by infusion of cochineal with the same salt.

“In cases of this kind, where the greatest possible economy, or even parsimony, of material is needful, I would advise the following mode of procedure for proving and corroborating your proof of the existence of blood, so that its presence in a stain may be affirmed with *absolute certainty* :

“From a suspected blood-spot upon metal, wood, leather, paper, muslin, or cloth, scrape with a fine

sharp knife two or three, or more, minute particles of the reddish substance, causing them to fall near the middle of a large thin glass cover. Apply in close proximity to them a very small drop of three fourths per cent. salt solution, bring the particles of supposed blood clot to its edge, and proceed as I have already directed.

“After thus examining the spectrum of the substance, you may generally, by rotating the stage, cause the coloured fluid to partly drain away from the solid portion, wherein, under favorable circumstances, should the specimen be blood, the granular white blood-globules become plainly visible, as do also cell-walls of the red disks. Among the latter, if your mental and physical vision is keen enough, you can, by the aid of a $\frac{1}{25}$ th-immersion lens and an eyepiece micrometer, measure a series of corpuscles accurately enough to discriminate human blood from that of an ox, pig, horse, or sheep.

“Lastly, to make assurance triply sure, lift up the thin glass cover, wipe off the tiny drop of blood solution and clot you have been examining on the folded edge of a thin piece of moistened blotting paper, let fall upon it a little fresh tincture of guaiacum, and then a drop of ozonised ether, which will at once strike the deep blue colour of the guaiacum test for blood. In this way I have actually obtained these three kinds of evidence, to wit, that of spectrum analysis, that of the microscope, and that of chemical reaction, from one single particle of blood, which, judged by a definite standard, certainly weighed less than one fifteen-thousandth, and

probably less than one twenty-five-thousandth of a grain."

Dr Richardson then goes on to criticise other methods, but the reader will not fail to notice that he goes wide of the mark, as he has to bring in the aid of the microscope while professing to have found an improvement over Mr Sorby's method of detecting blood by *means of the spectroscope*. I would advise those who are engaged in such research to follow Mr Sorby's advice, as his methods were arrived at after prolonged and careful study; indeed, he never lays down rules without good reason, and he may well be considered the greatest living authority in this special branch of inquiry.*

* In most cases I believe the blood-stained cloth could be made to yield hæmatin, either acid, or alkaline in a very simple way; thus, by digesting it in alcohol containing sulphuric acid, acid hæmatin may be obtained, or in alcohol containing ammonia, that variety of alkaline hæmatin before described would be obtained. By adding sulphide of ammonium to the latter fluid the bands of reduced hæmatin would appear, although there might be but the slightest trace of hæmatin in solution.

CHAPTER VII.

ABSORPTION SPECTRA OF BILE, URINE, ETC.

Brief sketch of the chemistry of the bile.—Before describing the spectra yielded by human bile when treated with reagents, or those of the bile of other animals, a few words on the chemistry of this fluid may not be uninteresting; moreover, a knowledge of its chemistry is absolutely necessary before commencing the study of its spectra.

The bile of man, of carnivorous and omnivorous animals, is “a bright golden red;” of graminivorous animals “a golden green, or a bright green, or dirty green,” but its colour in various animals will be referred to again. The reaction is alkaline. According to Frerichs the following is the average composition of human bile in 1000 parts :

Water	859·2	
Bile salts	91·4	} 140·8	
Fats, &c.	9·2		
Cholesterin	2·6		
Mucus and pigment	29·8		
Inorganic salts	7·8		_____
							1000·

Of these constituents, we are here concerned only with the pigments and the bile salts.

The colour of human bile is said to be due to *Bilirubin*,* a pigment which is also found in gall-stones, in jaundiced urine, and which can be prepared by the following method :—" Extract some powdered ox gall-stone successively with water, alcohol, dilute hydrochloric acid, boiling alcohol, and ether; then boil the dry powder with chloroform, and exhaust with this agent. Distil the chloroform from the red solutions, but not quite to dryness. To the residue add several volumes of absolute alcohol, and let stand twenty-four hours. There will be deposited a brilliant red powder mixed with steel-blue or brown crystals. Both the powder and the crystals are pure bilirubin, and can be separated by levigation with much absolute alcohol."† Human gall-stones, by this treatment, will, after the extraction of the bilifuscin, also yield bilirubin, but in very small quantity.

By exposing an alkaline solution of bilirubin to the air it turns green, and is converted into *Biliverdin*, the green pigment found in herbivorous bile (?).

Bilifuscin, another colouring matter, can be obtained from brown human gall-stones, by powdering the stone and extracting with ether, which removes the cholesterin. The powder is then treated with water and a little hydrochloric acid, and washed to neutrality. It is again extracted with boiling ether to remove fatty acids, and the powder boiled with absolute alcohol. Bilifuscin, will form a brown solution, and remains after evaporation of the alcohol as a black, shining, brittle mass, or as a dark brown powder.‡

* Thudichum, 'Chemical Physiology,' 1872.

† Ibid.

‡ Ibid.

The *salts* consist of sodium glycocholate and taurocholate. In ox-gall sodium glycocholate is abundant, and taurocholate scanty, while human bile contains chiefly sodium taurocholate, with a small quantity of the other salt. The bile of the cat, dog, bear, and other carnivora, contains only sodium taurocholate.*

This brief statement will recall to the reader's mind all that will be necessary to enable him to understand what follows.

Of the spectrum of bile itself.—Human, or dog, or cat bile gives no spectrum whatever† when it is fresh, but when it begins to decompose, or when extracted with alcohol it gives a spectrum, which will be described again. The bile of the lower animals—with a few exceptions besides those mentioned above—gives a spectrum which in some cases is a rather complicated one, and in most is more or less independent of the colour of this fluid. Although none of the bile-pigments mentioned above give any spectrum, there are other colouring-matters got from bile by treatment with stronger reagents than those required in the separation of bilirubin, biliverdin and bilifuscin, which, as the study of their spectra throws considerable light on what is to follow, will now be described. The pure chemical substances obtained by different observers from the oxidation of the bile-pigments must be mentioned first,

* We also find in the bile of the pig two peculiar acids united with sodium, viz. taurohyocholic and glycohyocholic. Again, goose-bile contains taurochenocholic acid.

† Although no spectrum is seen when the bile itself is examined, yet by careful dilution we can generally bring out a shading at F.

‡ Nor do Bilifuscin, Bilirubin, or Biliverdin.

and afterwards an account of the spectra which can be obtained by acting on bile itself with reagents, and of various kinds of bile, will follow.

Bilicyanin.—Maly, Heynsius, and Campbell have shown that bilirubin and other pigments treated with *oxidizing agents* yield a blue pigment, which, on account of its colour, has been named *bilicyanin*. This pigment is produced by mild oxidation, while by stronger oxidation *choletelin* is produced.

Bilicyanin was obtained by adding an alcoholic solution of bromine to bilirubin suspended in chloroform. As soon as the liquid assumes a blue colour it is left to evaporate, when there remains a substance which appears dark green when spread in a thin layer on porcelain, and which when dissolved in alcohol exhibits a fine blue colour. It is partially soluble in ether, and more so in a mixture of ether and alcohol. The alcoholic solution, on gentle heating with nitric acid, changes from blue to violet, then to purple red, and lastly to light brown; caustic potash causes it to assume a dingy sap-green colour, which turns to blue on the addition of hydrochloric acid; ammonia changes the blue solution to indigo-blue, which becomes a bright blue on adding hydrochloric acid. Sulphuretted hydrogen forms with bilicyanin in solution at first a bright green solution, and then flocks of biliverdin are precipitated, after the separation of which the liquid becomes colourless.

As two or three different kinds of spectra are yielded by bilicyanin characterised by the appearance of bands

* Or cholophaeine.

in the yellow and green, it cannot be considered a perfectly definite product. It is not found in bile-pigment without exposure to the air, or until the pigment has been oxidized in some other manner, but it exists in gall-stones and probably in urine, since hydrochloric acid acts upon it in the same manner as it acts upon indican, for which substance it has probably been mistaken in the urine.

Choletelin.—By passing nitrous vapours into alcohol in which bilirubin is suspended, this end-product of the oxidation process is obtained : by pouring the alcoholic solution in water after being thus treated, nearly all the colouring matter separates in the form of flakes, which dry up to a brown powder. This is soluble in alcohol, ether, and chloroform. It does not give any play of colour with nitric acid, but it has a more constant spectrum than that of bilicyanin ; thus, when in acid solution it gives one broad band extending from *b* to a little beyond F ; in an alkaline solution the band is less refrangible. It resembles thus both in its spectrum, and in the changes by acids and alkalies which are caused by the latter in the spectrum, somewhat the urobilin of Jaffé, which will be referred to further on.

Another blue colouring matter, which is probably a constituent of the bile itself, is described by E. Ritter.* It is not, like the last, produced by oxidation. It is found in the bile of man, the ox, sheep, pig, dog, and cat. He prepared it by shaking bile with chloroform until a yellow solution formed, and this was

* 'N. Rep. Pharm.,' xx, 569.

treated with sodium carbonate until colourless. It was then neutralised with hydrochloric acid, when two strata formed, one containing the yellow chloroform solution, the other the blue colouring matter in a state of suspension. The latter is insoluble in acids and chloroform, soluble in alkalis, forming a colourless or yellow solution, which, when exposed to the air, forms a brown precipitate, which after some time again becomes blue. In this respect it differs from reduced indigo, which turns blue when dissolved in alkalis and when exposed to the air.

Some bile-pigments described by Thudichum which give a spectrum.—In addition to choletelin and bili-cyanin Dr Thudichum describes, in the report quoted before, some other colouring matters produced by the action of various acids on bile which gave well-marked spectra: among these may be mentioned, first, one which was obtained by the action of concentrated nitric acid on an ammoniacal solution of bilirubin, when a blue precipitate was formed, which, after being quickly isolated by filtration and washing with water, was dissolved in alcohol. It showed an absorption band in yellow, and was called by the discoverer *cholocyamine*. Its spectrum is represented in Chart III, Sp. 18. By the action of fuming sulphuric acid on bilirubin he also obtained a sulphate of cholocyamine having much the same spectrum.

Two other bodies, *sulpho-cholocyamine* and *cholothalline*, were obtained by the action of sulphuric acid on bilirubin, but their consideration is of such little importance that they need not be further mentioned;

their description will be found in p. 252 of Thudichum's Report. *Cholonematine*, another substance, was obtained by the same observer from the alcoholic extract of the colouring matter of human gall-stones, which, on account of its remarkable spectrum, is figured in Chart III, Sp. 19.* This is the spectrum of an ethereal solution, which appeared green in reflected light, and "brown in transparent dilute solution." *Boviprasine*, a green colouring matter, giving the spectrum shown in Chart III, Sp. 20, was obtained from the alcoholic extract of the gall-stones of the ox; the spectrum was that of an ethereal solution of the resinous residue left after evaporation of the alcoholic solution.

Hyocœruline is a blue matter obtained by the same observer from the gall-stones of a pig, the spectrum of which, dissolved in alcohol, is shown in Chart III, Sp. 21. Another colouring matter from the same source is called *hyoflavine*, which, when dissolved in alcohol and potash, boiled, and nitric acid added, gave a blue solution, showing two bands and a third broad and dark band. This spectrum is shown in Chart III, Sp. 22. My object in figuring these spectra is for the sake of comparison, *e.g.* compare the last spectrum with Chart II, Sp. 5, which latter was got by acting on human-bile solution with nitric acid, or with pig-bile solution acted on by the same acid, when almost the same spectrum is obtained. There are various other bile spectra described and figured by Thudichum, but they are not of sufficient medical interest to call for their being mentioned here.

* Copied from Plate IV of 'Report.'

Spectra obtained from the bile of some of the lower animals.—I have been engaged for a considerable time in investigating the spectrum of the bile in various animals, and as this has not been done by others, except Dr Dalton, of New York, at least, so far as I have been able to ascertain, I shall now give the results at which I have arrived. The animals whose bile has been examined are :

- | | | |
|----------------|----------------|----------------|
| 1. Man. | 7. Mouse. | 13. Chicken. |
| 2. Pig. | 8. Sheep. | 14. Goose. |
| 3. Dog. | 9. Hedgehog. | 15. Wild duck. |
| 4. Cat. | 10. Ox. | 16. Duck. |
| 5. Guinea-pig. | 11. Crow. | 17. Frog. |
| 6. Rabbit. | 12. Blackbird. | |

Among these animals the bile of the following gave a characteristic spectrum :

Guinea-pig.	Mouse.	Ox.
Rabbit.	Sheep.	Crow.

It is a curious fact that the darkest green or golden-red bile gave the least characteristic spectrum. The carnivorous and omnivorous bile gave a negative result with slight exceptions; that of the herbivora and graminivora gave a characteristic one, while the insectivorous specimen was negative among the mammals; and the bile of the frog, which was the only reptilian one examined, showed a resemblance to the mammalian insectivorous specimen. Among the birds the opposite fact was noticed. While those birds, such as the blackbird, chicken, goose, wild duck, and

duck, gave nothing very remarkable, yet the bile of the crow, which may well be considered an omnivorous bird, gave a most characteristic spectrum.

There is one feature in common, viz. that by careful dilution a band can, in almost every specimen of bile, be made to appear at F, which is intensified by acids, and made almost, or altogether, to disappear by alkalies, besides the play of colours with nitric acid which all specimens of bile give.

Human bile, as was referred to before, gives no spectrum,* except that by very careful dilution we can generally bring out a shading at F, which shading is intensified by hydrochloric acid and diminished by caustic alkalies.

Pig-bile, when fresh, is said to give no spectrum; four hours after it had been removed from the gall-bladder the fluid itself appeared brownish red in deep, and yellow in thin, layers, and gave a feeble absorption band, which is shown in Chart II, Sp. 6.

Dog-bile, obtained fresh from the gall-bladder, was a golden-brown colour, and gave the feeblest possible shadow between D and E, but no well-marked band. On examining this bile in thinner and thinner layers it gave no other bands.

Cat-bile, obtained perfectly fresh, was of a golden-yellow colour with a greenish tinge; in deep layers (5 mm.) no bands were visible, but in a thin layer a band was seen, which read on my scale from 46 to 55, so that it was placed at F, which it covered. The

* When old, or when extracted by alcohol, it gives a band at D. *Vide antea*, p. 151.

band of urobilin, Chart II, Sp. 16, will represent its position. This band was intensified by treating solutions of the bile with hydrochloric acid, and diminished by caustic potash, &c.

Bile of the guinea-pig, obtained fresh; its colour was golden yellow, and it was free from blood. It gave the spectrum shown in Chart II, Sp. 10. By dilution, or by thinning the layer, a feeble shadow could be seen at F.

Bile of the rabbit.—Freshly examined its colour was sap-green in thin layers. It showed at a suitable depth three bands, which are seen in Chart II, Sp. 11.

Bile of the mouse.—Fresh; yellow in colour.—It gave, when examined in a thin layer or in solution, a splendidly marked band at F, which was made still darker by adding mineral acids, and diminished by caustic alkalis, so that it would appear the colouring matter of the bile of the mouse is identical with the bile-pigment called urobilin,* constantly present in human urine.

Bile of the sheep and ox.—When obtained fresh it is green, but soon changes to reddish brown, and presents exactly the same spectrum when obtained from the ox that it does when it is got from the sheep. This spectrum is a very fine one, and presents in a deep layer three bands, in a thinner one four bands, and in a still thinner a fifth band at F is visible. Chart II, Sp. 7, shows the spectrum of the four bands; the fifth may be represented by that seen in Sp. 8, which is the spectrum of this fluid treated by nitric

* See the account of its spectrum, *infra*.

acid. Sp. 9 is that of a solution treated with hydrochloric acid, the precipitate of cholic acid, &c., being in both cases redissolved in boiling alcohol.

Bile of the hedgehog.—This bile, obtained fresh from the gall-bladder, was a fine blue-green colour. It gave no absorption band, but a feeble shading was noticed at F.

Bile of the crow.—Of a yellowish-green colour; it gave a very remarkable spectrum, which is shown in Chart II, Sp. 13. The band in the extreme red is best seen by lamp-light.

Bile of the blackbird.—Of a yellowish colour; gave only a shadow at F.

Bile of the chicken.—The colour was dark sap-green; at first no band was visible, but after having been exposed to the air for a few minutes the usual band at F appeared.

Bile of the goose.—Colour dirty green; there was no band except a shadow at F.

Bile of the wild duck.—Sap-green in colour; this bile gave only the band at F.

Bile of the domestic duck.—It was of a dark sap-green colour; but gave no characteristic spectrum except a band at F.

Bile of the frog.—From the colour of the bile of this animal, which was a fine bright green, one would be led to expect a characteristic spectrum, but, with the exception of the shading in the red and violet ends of the spectrum, and an uncertain shading at F, there was nothing remarkable.

Remarks on the above spectra.—The absorption band

at F thus appears to be the link which binds all these specimens of bile together, and which is seen with the same distinctness in the bile of the mouse as it appears in febrile human urine. Thus, the examination of the bile of an insignificant little mammal has thrown light upon an obscure fact in physiological chemistry, a statement with which the reader will agree when he has read what follows.

The spectrum of Gmelin's reaction.—When to a solution of bile in a test tube we add strong nitric acid the solution at once undergoes a change of colour, becoming green, blue, violet, red, and lastly yellow or brownish-yellow, and if the experiment has been performed by pouring the nitric acid into a test tube before the slit of the chemical spectroscope we notice a broad shading (probably composed of two distinct bands) in orange and yellow, and a black band extending from near *b* to beyond F; this is shown in Chart II, Sp. 5. In a very short time the shading in orange begins to fade, and at the time the oxidation-process is completed and the colour of the solution has become yellow, nothing but the band at F is left.

Or the experiment may be varied in this way:—A little of the bile itself may be put into a glass cell, nitric acid added, and boiling alcohol then poured into the cell, which has the effect, not only of dissolving the precipitate, but of arresting the oxidation process for a short time, and thus enables any particular colour to be observed. On examining such a solution we get, if we have acted on human bile, the spectrum shown in Chart II, Sp. 5, and we may by

adding more and more alcohol cause the disappearance of the band in orange until, as before mentioned, that at F only is left.

Ox bile treated in the same manner gives the spectrum shown in the same chart, Sp. 8, or treated with hydrochloric acid, Sp. 9.*

This spectrum teaches that many colouring matters derived from bile were actually formed by reagents upon that fluid, and to me it appears that bilirubin, and perhaps bilifuscin, in *carnivora*, biliverdin (when it will have been properly obtained) in *herbivora*, &c., and the so-called urobilin, are the only bile-pigments which are normally present. The reaction of nitric acid upon bile-pigment has been studied by Jaffé,† who states that the different colours produced correspond to characteristic alterations of the spectrum; in fact, his description‡ corresponds with what has been already said. He, however, succeeded in isolating the pigment present in the blue-violet solution, by treating an alcoholic solution of biliverdin or an ammoniacal solution of bilirubin mixed with alcohol with a mixture of ordinary concentrated nitric acid, until a sample showed the bands on each side D, the solution being then mixed with chloroform and shaken with water. The water was then removed, the chloroform layer containing the blue pigment was filtered from the

* If pig-bile is treated in this way, we get a band on each side D, separated by an interval of yellow colour, which may be compared with Thudichum's oxidation product from hyoflavine, Chart III, Sp. 22.

† 'Zeitsch. f. Chem.,' v, 666.

‡ When I first described this spectrum in 1877, I was quite ignorant of Jaffé's researches on 'Gmelin's Reaction.'

biliverdin and evaporated to dryness, and the residue purified by repeated solution in chloroform. The pigment thus obtained is of a deep violet colour, insoluble in water, soluble in alcohol, ether, and chloroform, forming solutions of a violet colour. Alkalis dissolve it, the solutions being brown-violet; acids also form solutions with it of a blue colour. The neutral and alkaline solutions exhibit no absorption bands, but a small trace of an acid causes the appearance of the original spectrum.

Urobilin.—Jaffé also isolated the pigment which gives the band at F, having obtained it by the same process as in the last case. The substance which was got was brown red, soluble in alcohol, ether, and chloroform, the solution being a fine red colour, unaltered by acids or alkalis; but the acid solution *only* exhibited the original absorption band.

By the action of hydrochloric acid on the bile of the dog, the same observer obtained a red solution which gave exactly the same band. This red solution becomes yellow on adding alkalies to it, and then gives another band between C and F, but nearer to C.* Chloroform extracts the colour from the solution, and on evaporating the chloroform a red residue is left, soluble in water, in alcohol, and in chloroform. It can be precipitated from the aqueous solution by lead acetate or calcium chloride.

The great pathological importance of this discovery of Jaffé's cannot be over-estimated, for the pigment which he succeeded in isolating from the bile of the

* Watts' 'Dict.,' 1st Supp. Is not C a misprint for E?

dog, is identical with the urobilin which I have found in every specimen of human urine. A substance giving the same spectrum may be obtained from human bile by the simple addition of hydrochloric acid to a solution of the former.

Detection of bile-pigment in urine by Gmelin's test and its spectrum.—By means of the spectrum described above we can be certain that the colour reaction developed on the addition of nitric acid is due to bile-pigment; the appearance of a shadow on each side D, which soon disappears, being absolute proof that bile-pigment is present. This shows the importance of using the spectroscope in clinical work, since the colour reaction *alone* is attended with difficulties, which are considerably diminished by using this instrument.

Reducible product of the oxidation of bile-pigment.—According to Stokvis,* “this substance is formed as a secondary product in most cases of the oxidation of biliary colouring matter whereby Gmelin's reaction is produced. It is colourless, or of a light yellow tint, and is soluble in water, alcohol, and dilute acids. It becomes of a beautiful rose-red colour when boiled with reducing agents in alkaline solutions. The red solution gives in the spectrum a broad band in green. In thick strata or concentrated solutions the band begins close to D and extends to *b*. In dilute solutions (thin strata) it occupies only two thirds of the distance between D and E, ending short of E. Shaking with air causes both the rose colour and the band

* “N. Rep. Pharm.,” xxi, 123, Watts' ‘Diet.,’ 2nd Supp., 1875.

to disappear. This by-product differs from the bile-colouring matter and other oxidation products of the same, in being insoluble in chloroform and ether, and in not forming insoluble compounds with neutral or basic lead acetate. It is precipitated, however, by ammonia and basic lead acetate.

“This substance exists as such in the gall-stones of man and of the ox, and it can be obtained from them by boiling with distilled water and extracting with dilute acids. It does not exist in fresh bile. It occurs in the urine of animals which has been standing for some days previously, in jaundiced urine, and in the urine of febrile diseases, *e.g.* smallpox, typhus, &c. *It is not found in healthy urine.* It seems to be present in the alimentary canal, although in direct experiments with different kinds of food little or none could be found. In alkaline solutions it soon loses its characteristic properties. Its occurrence in any liquid of neutral or acid reaction affords an indication of the previous existence of bile-pigments therein. In applying the test, the liquid is to be precipitated with lead acetate, excess of lead removed by oxalic acid, and the filtrate concentrated and boiled with alkalies and a reducing agent. If no reduction takes place, and if the other tests for biliary colouring matter have given a negative result, their absence may be safely inferred.”

This is probably the colouring matter which I have found several times in various morbid urines on adding to them strong nitric acid, and accordingly its description has been fully given; this fact will be referred

to when the spectra of these specimens of urine are described.

The spectrum of Pettenkofer's test for bile acids.— I have in a former paper* described Pettenkofer's test as consisting of two bands, but I now find that the band at F must have been due to the action of sulphuric acid on the bile-pigment. In Chart II, Sp. 14, is a representation of the spectrum, which was obtained as follows:—Human bile was treated with absolute alcohol and filtered; the filtrate was thoroughly decolorised with animal charcoal, and Pettenkofer's test was applied with every possible precaution to a small portion of the alcoholic solution; it gave two bands nearly in the same position as those represented in Sp. 14, which is the spectrum of the aqueous solution of the bile salts obtained by evaporating off the alcohol and dissolving the residue in water. I then examined the spectrum of Pettenkofer's test as applied to the bile salts of the pig, the solution being prepared as before, and the result was the spectrum shown in Chart II, Sp. 15. The band near D in Sp. 14 is hardly, if at all, visible in the chemical spectroscope, and is probably of little consequence. This spectrum enables us to distinguish the purple reaction given by the action of sulphuric acid and sugar on bile salts from the reaction developed by the same reagents with many other bodies, but I fear its use in examining for

* 'Dub. Journal,' 1877. According to Heynsius and Campbell, the colour developed by the action of sulphuric acid and sugar with sodium taurocholate gives a spectrum of three bands, one between C and D, the next between D and E, and the third between b and F. Pfluger's 'Archiv. f. Physiol.,' iv, 497.

them in urine is exceedingly limited for obvious reasons. Thudichum's map of Pettenkofer's reaction is shown in Chart III, Sp. 16.

According to Thudichum the salts are absent from bile in certain "diseased conditions" of this fluid, and in fatal cases of cholera.*

Urobilin in human urine.—In the concluding part of this chapter the importance of a knowledge of the researches of Jaffé on bile-pigments will be shown, in enabling us to understand the nature of the colouring matter present in urine in health; and the discovery of the other oxidation product of bile-pigment by Stokvis is important, since it throws light upon a spectrum which is seen in urine in certain pathological conditions when this fluid is treated with nitric acid.

The accusation of being a "test-tube chemist" may be brought against any one who accepts Jaffé's views, but the test-tube has done a great deal for medicine, and in this instance, assisted by the spectro-scope, it has enabled us to understand a subject which will be of the greatest benefit to clinical medicine, and which, when it has been sufficiently worked out, will be of immense value both in diagnosis and in treatment.

It would be beyond the scope of this little volume were I to give an account of all the pigments which have been separated from urine; it will suffice to say that there is only one pigment which gives a well-marked absorption band, and there are two propositions which may be laid down with regard to this pigment:

* 'Chemical Physiology,' 1872, p. 74.

I. *That normal human urine always contains a pigment, the spectrum of which in acid solution is characterised by an absorption band at Fraunhofer's line F.*

II. *This absorption band behaves on treatment with reagents in the same manner as that of the substance obtained by the action of hydrochloric acid on bile by Jaffé, and like that obtained by Maly from bilirubin, which he called hydrobilirubin, so that we may conclude that the substance in solution which gives the band at F is urobilin.*

I do not maintain that urine owes *all* its colour to urobilin, as there are no doubt other pigments present as occasional ingredients, but there is no doubt whatever that urobilin is constantly present in every specimen of human urine in health, that when this fluid is high coloured the absorption band at F is dark, that when it is not high coloured then the band is feeble, and it may not be visible in very alkaline urine. That it is present in the last case can be demonstrated, however, by the addition of nitric, or of hydrochloric, or even acetic acid, when it appears at once. Whatever the pathological significance of the other pigments that have been described may be, urobilin is the pigment which ought to have the greatest attention paid to it by clinical observers, for although constantly present in *healthy* human urine it is sometimes absent in disease. I am not at present in a position to say in what diseases it is absent, but that it is so is a fact beyond dispute.

The spectrum of normal human urine of acid reaction is shown in Chart II, Sp. 16.

This band is especially well marked in some febrile urines, but it is not quite correct to say that caustic potash and caustic soda cause it to be replaced by another one, for although this does sometimes happen in the urine of disease, it is not the case in healthy urine. The fact—that acids intensify the band and that alkalis, more especially caustic potash and ammonia, cause it to disappear, constitutes the test which distinguishes urobilin from other pigments which give an absorption band in the same part of the spectrum.

Urobilin in pink urates.—A hot alcoholic solution of pink urates gave the band of urobilin with remarkable distinctness, intensified by acids, and disappearing on the addition of alkalies.

Another bile-pigment in the urine of disease.—Besides urobilin, the spectroscopist teaches that another bile-pigment appears in certain morbid conditions in the urine upon the addition of nitric acid; it is indicated by an absorption-band which occurs on the red side of the band of urobilin; it is probably that discovered by Stokvis, which was mentioned in page 163. It was found to occur in the following specimens of urine :*

(1) Urine of rheumatic fever; patient being treated by salicylate of soda (10 grains *ter die*). The colour of the fluid was a light yellow; reaction acid; perchloride of iron in solution developed the usual salicylate reaction; no albumen. On examining the urine itself a band in the position of that of urobilin appeared;

* In none of the specimens could the colour-reaction known as Gmelin's test be developed.

on adding nitric acid the colour of the fluid became slightly pink or lavender-pink, *and it now gave another band*, extending from about half way between D and E to near E; the urobilin band was still visible (Chart II, Sp. 17). The urine from the same patient, and taking the same drug, failed to give this reaction three days after the last examination. The salicylic acid had nothing to do with the appearance of this absorption band, as another specimen of urine from a case of phthisis treated with the salicylate failed to give the band; nor is it constantly present in rheumatic fever urine, as several specimens from other cases were examined with a negative result.

(2) The urine of a case of pregnancy (6th month) when treated with nitric acid developed a band nearly in the same position, in addition to that of urobilin, but it is not constantly present in the urine of pregnant women.

(3) The urine of a case of thoracic aneurism accompanied by albuminuria; dark orange yellow in colour. It gave the spectrum of urobilin, but when treated with nitric acid a dark absorption band made its appearance, *in a different position* from that of the first specimens. This will be observed if Sp. 18, that of this specimen, is compared with Sp. 17 (Chart II). Hydrochloric acid only intensified the urobilin band, but caustic potash when added to the original urine at first caused the disappearance of the urobilin band, which was soon replaced by another nearer the red (see Chart II, Sp. 19). Another specimen of urine obtained some days after this examination

from the same patient behaved in an exactly similar manner.

(4) The urine of a case of cirrhosis of the liver, of a dark straw colour, gave a spectrum almost identical with that shown in Sp. 17.

(5) From a case of cancer of the pylorus, the urine, which was a straw colour, gave a spectrum closely resembling the last in the presence of two bands, but that near the red was a little nearer *b* than in the last case.

Out of some hundreds of specimens of urine, from patients suffering from trifling ailments, which I have examined with the spectroscope I have never got a second absorption band. That which was seen was always due to the presence of urobilin only. Probably the appearance of this second band indicates grave disturbance in the system, as it appears only in those cases where there is undoubted disease of a severe character, with the exception of pregnancy.*

Connection between the colouring matters of blood, bile, and urine.—Though such a connection is denied by some chemists, who assert that it is impossible that bile-pigment can be derived from hæmoglobin, there is undoubtedly a time approaching when convincing proof of this fact will be forthcoming, and also of the no less important one that the colouring matter of urine is derived from bile-pigment; mean-

* The presence of urobilin is sometimes difficult of detection; but by examining the urine in a deep layer, or by adopting other precautions which for that particular case will suggest themselves, its band can always be seen.

time we may believe that what have been supposed to be the true colouring matters of bile and of urine are not such, but probably a mixture of several substances, owing to imperfect methods of separation. The spectroscope has suggested the connection, and when our knowledge of chemistry will be precise enough to allow of our being able to follow up the hints it has given, controversy will be no longer possible.

Bilirubin is now generally supposed to be formed from hæmoglobin, though actual proof has not yet been forthcoming. There is no doubt that hæmoglobin in its passage through the liver is actually converted into bilirubin, and there is, according to some, an identity between hæmatoidin and bilirubin (though this is denied by others). It has been affirmed by some that bile-pigments have appeared in the urine after the injection of hæmoglobin solution into the veins, or of any substance which is capable of dissolving the blood-corpuscles and setting free hæmoglobin, such as water, bile acids, or ether; but whether this has been proved or not, there is no doubt that urobilin can be formed from bilirubin.

R. Maly* has found that by dissolving bilirubin in dilute soda- or potash-ley, and adding sodium amalgam, the air being excluded, no hydrogen was given off, but the dark colour gradually lightened, and after two or three days' action the solution acquired a yellow or bright brown-yellow colour, and then gave off hydrogen. From this liquid hydrochloric acid sepa-

* 'Ann. Ch. Pharm.,' clxi, 368; clxiii, 77.

rated a pigment, which appeared to be a weak acid, yielding with alkalis brown-yellow soluble salts, and with heavy metals insoluble compounds, which separated in red flakes. It was soluble in alcohol, slightly in water, readily in ammonia and fixed alkalies, in ether, liquid hydrocarbons, glacial acetic acid, and chloroform. Its spectrum is the same as that of urobilin, the band being intensified by acids and decreased by alkalis; this has been called hydrobilirubin, but it differs in no respect from urobilin. It is also found in fæces. Biliverdin can also be made to yield it in the same way. (See Thudichum's 'Annals of Chemical Medicine.')

The detection of blood, bile-pigment, and bile-acids in urine by means of the spectroscope has been already referred to. Sugar and albumen cannot be detected by this instrument, but we have already sufficiently delicate tests by means of which the smallest quantity of either can be detected with certainty.

Urocyanine and urorubine.—The urine from cholera patients in the early stage of reaction when cautiously boiled with nitric acid often gives a blue colour and sometimes a blue deposit. This deposit is soluble in alcohol, forming a dichroic purple-blue solution, giving a broad absorption band in yellow ("and green?"), Chart III, Sp. 23 (Thudichum).*

Lutein.—"Luteine" is the name given by Dr

* The absorption band described by Dr Moss in the 'Medico-Chirurgical Transactions,' 1875, which he found in the urine of a case of cirrhosis of the liver is nothing but the band of urobilin. This spectrum is referred to in Beale's 'Microscope in Medicine,' last edition, p. 501, but the author of that book did not notice the error.

Thudichum* to a substance which he first discovered in the juice of the corpora lutea of mammals. To prepare it he dissected out the corpora lutea from the ovaries (of cows), pounded, warmed them, and pressed out the juice. The alcoholic solution of this dried residue gave the spectrum shown in Chart III, Sp. 10. This solution was examined by means of lime light. The etherial and chloroformic differ slightly in the position of the bands in their spectra from the alcoholic solutions.

By boiling the yolks of eggs in 85 per cent. alcohol, filtering, and allowing to stand till clear, we get a solution giving nearly the same spectrum (this is ovoluteine).

Butyro-lutein is got by digesting butter in chloroform and filtering, when we also get three bands nearly in the same place.

Cysto-lutein.—The same substance was discovered by Thudichum in ovarian cysts by means of the spectroscope, which showed bands in the blue, nearly in the same position as the bands of ovario-lutein got from cows' ovaries. (See p. 130.)

Intestino-lutein was got from the fæces of sucking infants by mixing the fæces with alcohol, and filtering from the flakes of caseine. It gave a band between *b* and *G* and covering *F*. (Chart III, Sp. 13.)

Sero-lutein in blood-serum and in pathological fluids.—By allowing blood to stand, decanting off

* See Appendix and 'Chemical Physiology.' Piccoli and Lieben first described the yellow substance giving this spectrum according to Watts, 2nd Supp.

the serum, allowing this to deposit, pouring off the supernatant liquid, and filtering repeatedly until clear, we get a fluid which gives one band at F and a very faint one between F and G. The importance of this to pathology is shown by the fact that I have found this absorption band at F in fluid removed from the pleural cavity by aspiration, and also in peritoneal fluid from a case of ascites due to chronic peritonitis.

The spectrum of sero-lutein from dog's blood is shown in Chart II, Sp. 24, and in Chart III, Sp. 12, and the same band from the ascitic fluid in Chart II, Sp. 23. Perhaps the question may be asked, Was not the band due to urobilin? If it had been it would have been intensified by acids and removed by alkalis, but the latter seemed rather to darken it, as did also the addition of ammonium sulphide. The same band was also noticed in sero-purulent fluid removed from an abscess.

Spectra yielded by fæces.—Vaulair and Masius obtained urobilin from fæces, and I have myself noticed the band of this substance in meconium. The analysis of Zweifel* would lead us to expect its presence in the latter. It is probably formed in the intestine from bilirubin by the same process as it was prepared by Maly, inasmuch as abundance of hydrogen is present in the intestine, and immediately exerts a hydrogenising action. By boiling fæces with sulphuric acid Thudichum obtained a substance, the spectrum of which is represented in Chart III, Sp. 14. This may be compared with Sp. 15, from treating a

* 'Archiv. für Gynäcologie,' Band vii, p. 474.

cholera stool in the same manner, and is very like sulphate of cruentin, shown in Chart I, Sp. 16.

Other physiological and pathological spectra.—The absorption spectra which have been described are those which will be found most useful to workers with the spectroscope; there are three more which will require to be merely mentioned.

Murexide, the purple colour produced from uric acid by the action of nitric acid and ammonia, gives a broad absorption band extending from D to F. (Thudichum.)

Fluopittine, a body obtained by Thudichum by the decomposition of albumen, gives the complicated spectrum shown in Chart III, Sp. 17.

Liquor amnii gives a faint shadow at F, probably due to lutein.

There is no doubt that when the spectroscope comes to be used more extensively than it has been hitherto, a great number of pathological absorption spectra will be discovered; and it remains for those who have abundant clinical opportunities to do their share. By adding another exact physical method to their means of diagnosis, they will help to make medicine approach more nearly to the position which we all hope it will some day occupy—that of an exact science.

APPENDIX I.

WAVE-LENGTHS.

MR SORBY has, after having introduced a method of printing spectra in formulæ, come to the conclusion that all results should be expressed in wave-lengths; and he does this with his own microspectroscope by means of a complicated apparatus, composed of a plate of quartz placed between two Nicol's prisms, the thickness of the plate being such that the whole spectrum contains twelve dark bands. He prefers this method to reducing his readings to wave-lengths by means of the bright-point micrometer, as he maintains the latter method "has unfortunately several serious defects for expeditious practical working;" but the adoption of Mr Sorby's apparatus is attended with even greater difficulties than any other. The photographed scale which I have had attached to my microspectroscope enables me to reduce the reading of any band to wave-lengths in a few seconds with the help of the curve I have described on p. 32, and after having used it for a considerable time I can recommend this method before any other. All we have to do (as stated before) is to take the reading of the centre of the band, find this number on the top line of the scale, and then its posi-

tion on the curve, the wave-length corresponding to the number will be found on the right-hand side of the scale.

The importance of the wave-length method is further increased by the discovery made by Mr Sorby, that there is "*a far more uniform connection between the wave-lengths of the centres of bands of the spectrum of a single substance containing a number of bands than there is between any other conditions.*"

Thus, in many spectra having a series of bands whose centres are at wave-lengths a, b, c , and d , there is the same *ratio* between each consecutive two, so that $\frac{a}{b} = \frac{b}{c} = \frac{c}{d}$. Again, in the case of substances giving two or more well-marked bands, though the actual wave-lengths of the centres of the bands may vary with the conditions in which the substance occurs (solid or in solution), yet the *ratio* between the wave-lengths of the bands remains almost, if not quite, constant. Thus, in yellow xanthophyll, which may be taken as an example, we have :*

Condition.	Centre of the two bands.	Ratio.
In free state and solid	501 469	1 : .936
In carbon bisulphide	498 467	1 : .937
In absolute alcohol	471 442	1 : .938
Combined with Canada balsam . .	488 457	1 : .936

* See the 'Monthly Microscopical Journal,' vol. xiii, p. 198.

EXAMPLE OF THE METHOD OF REDUCING READINGS TO
WAVE-LENGTHS.

An example will make the method of calculating wave-lengths by means of the curve shown opposite page 32 clear.

Thus, some stale urine containing blood due to the breaking down of a carcinomatous growth in the bladder, gives three bands (those of methæmoglobin). Now, on taking their readings on the scale attached to my microspectroscope, I find that the centre of the first band is at 17·5; I then find its place on the top line of the scale, and running my eye down the scale, I find where the line corresponding to this number and the curve intersect each other, and opposite to this point on the right-hand line of the scale I find the number 624. Therefore, the wave-length corresponding to the centre of the first band is 624.

The scale reading of the centre of the next band is 27, and on proceeding as before, we find its wave-length is 574.

The scale reading of the centre of the next band is 35·5, and its wave-length is therefore 540.

It must be distinctly understood that the numbers at the top of the scale will differ according to the method of measurement adopted by each observer, and in this case they are those of my own scale; but those at the right-hand line are constant. Having tried this method in every possible manner, I can recommend

its adoption to those who are anxious to reduce their readings to wave-lengths. In the lithographed scale the squares which represent those on the original are square centimètres, while the latter were square inches, the smaller divisions being square tenths of an inch.

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ADDITIONAL NOTE.

WHILE the last page of this book was passing through the press a small paragraph appeared in the 'Medical Times and Gazette' (Dec. 6th, 1879), headed "Poisoning by Chlorate of Potash," from which it appears that, according to Dr. F. Marchand ('Virchow's Archiv,' Bd. 27, Heft 3), chlorate of potash in poisonous doses causes the blood to assume a chocolate colour, this change of colour being due to the conversion of hæmoglobin into methæmoglobin, as evidenced by the spectrum. (Cf. the action of nitrites.)

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ERRATA.

Page 8, second line from bottom, for "burned in an iron spoon," read "placed in an iron spoon."

In Chart I, Sp. 19, there is some shading shown between the second and third bands; this is not correct.



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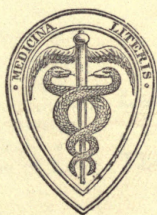
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